PATENT COOPERATION TREATY

REC'D 16 MAR 2001
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

3

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
992514wo Me/bk		
International application No. PCT/EP99/08744	**International filing date** *(day/month/year)* 12/11/1999	**Priority date** *(day/month/year)* 12/11/1998

International Patent Classification (IPC) or national classification and IPC
C12N15/12

Applicant
NITSCH, ROGER

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/05/2000	13.03.2001

Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Montero Lopez, B Telephone No. +31 70 340 3739

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/08744

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-33 as originally filed

Claims, No.:

1-38 as originally filed

Drawings, sheets:

1/29-29/29 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 21(g) and (h), 22(g) and (h), 23(g) and (h), 24-27 as far as dependent on 21(g) and (h), 22(g) and (h) and 23(g) and (h); 35 (g) and (h); 36 and 37 as far as depending on 35(g) and (h); 38(g) and (h); 28-34.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 21(g) and (h), 22(g) and (h), 23(g) and (h), 24-27 as far as dependent on 21(g) and (h), 22(g) and (h) and 23(g) and (h); 35 (g) and (h); 36 and 37 as far as depending on 35(g) and (h); 38(g) and (h); 28-34.

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/08744

1. Statement

Novelty (N)	Yes:	Claims	1-4, 6-27, 35-38
	No:	Claims	5
Inventive step (IS)	Yes:	Claims	1-4, 6, 7, 14-20, 24, 26, 27, 36, 37
	No:	Claims	5, 8-13, 21-23, 25, 35, 38
Industrial applicability (IA)	Yes:	Claims	1-27, 35-38
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: Emhum2 Database Entry Hsrsc390 Accession number D13643; 31 March 1993 NOMURA N.: 'Human mRNA for KIAA0018 gene, complete CDS.' XP002099607

D2: NOBUO NOMURA ET AL.: 'Prediction of the coding sequences of unidentified human genes. I. The coding sequences of 40 new genes (KIAA0001-KIAA0040) deduced by analysis of randomly sampled cDNA clones from human immature myeloid cell line KG-1' DNA RESEARCH, vol. 1, no. 1, 1994, pages 27-35, XP002099608

D3: NOBUO NOMURA ET AL.: 'Prediction of the coding sequences of unidentified human genes. I. The coding sequences of 40 new genes (KIAA0001-KIAA0040) deduced by analysis of randomly sampled cDNA clones from human immature myeloid cell line KG-1 (Supplement)' DNA RESEARCH, vol. 1, no. 1, 1994, pages 47-56, XP002065816

1. Claims 1-7, and 14-20 of the underlying application relate to a protein molecule named SELADIN-1, shown in SEQ ID NO:1, nucleic acid encoding it, shown in SEQ ID NO:2, and variants thereof having the function of protecting cells against degeneration and/or cell death, as well as antibodies immunoreactive with the protein.

1.1. No such sequences or variants thereof having the alleged function have been disclosed in the state of the art and therefore, claims 1-4, 6, 7 and 14-20 are novel and meet the requirements of Article 33(2) PCT.

1.2. Document D1, which is considered to represent the most relevant state of the art, discloses a polypeptide and encoding nucleic acid isolated from a human myeloid cell line which differs from SEQ ID NO:2 in lacking the first 62 nucleotides and lacking a C nucleotide in position 1228. This results in a frame-shift in the open-reading frame sequence which translates into a polypeptide lacking

aminoacids 391-516 of SEQ ID NO:1 and a sequence difference in aminoacids 377-390. No function for this gene, designated KIAA0018, or its encoded protein is disclosed. Due to the above mentioned differences in nucleotide sequence, the skilled person would not have been able to provide a polypeptide of SEQ ID NO:1 or its encoding nucleic acid or a variant thereof able to protect cells against degeneration and/or cell death, without exercising an inventive step. Consequently, claims 1-4, 6, 7 and 14-20 are considered to involve an inventive step and comply with the requirements of Article 33(3) PCT.

1.3. Claim 5 is directed to a DNA molecule capable of hybridizing with the complement of sequence SEQ ID NO:2. Document D1 discloses a polypeptide and encoding nucleic acid isolated from a human myeloid cell line which only differs from SEQ ID NO:2 in lacking the first 62 nucleotides and lacking a C nucleotide in position 1228. Due to sequence similarity between both DNA molecules it is considered that the nucleic acid of D1 would hybridize with the complement of SEQ ID NO:2. Consequently, claim 5 is not novel contrary to the requirements of Article 33(2) PCT.

2. Claims 8-13 refer to a vector and a cell comprising the nucleic acid encoding SEQ ID NO:1 or variants thereof. The subject-matter of claims 8-13 is considered novel and complies with the requirements of Article 33(2) PCT.

2.1. Document D1, which is considered to represent the most relevant state of the art, discloses a polypeptide and encoding nucleic acid isolated from a human myeloid cell line which differs from SEQ ID NO:2 in lacking the first 62 nucleotides and lacking a C nucleotide in position 1228. This results in a frame-shift in the open- reading frame sequence which translates into a polypeptide lacking aminoacids 391-516 of SEQ ID NO:1 and a sequence difference in aminoacids 377-390. The attention of the applicant is drawn to the fact that the term "variant" does not appear to have a precise meaning. In this light, it can be considered that the sequence disclosed in D1 is substantially a variant of SEQ ID NO:1, since they are identical through 376 aminoacids. Moreover, the insertion of a nucleic acid molecule into a vector and a host cell constitute standard embodiments in the art which the skilled person would regard as a normal application of the nucleic acid disclosed in D1. Thus, the subject-matter of claims 8-13 does not involve an

inventive step and does not satisfy the criterion set forth in Article 33(3) PCT.

3. Claims 21-27 and 38 relate to methods of diagnosing and monitoring a disease and evaluating a treatment for a disease by, among others, determining the level or activity of a molecule capable of hybridizing with the complement of SEQ ID NO:2.

3.1. Such methods as mentioned above constitute routine manipulations in the state of the art, which the skilled person would put into practice for the molecule disclosed in D1 without the need of exercising any inventive skill. Claims 21-23, 25 and 38 lack, therefore, inventive step and do not comply with the requirements of Article 33(3) PCT.

3.2. Claims 24, 26 and 27 involve the above mentioned methods specifying the function of the protein as protecting cells against degeneration and/or cell death. For the reasons already put forward in paragraph 1.2 above it is considered that claims 24, 26 and 27 involve an inventive step and comply with the requirements of Article 33(3) PCT.

4. Claims 35-37 encompass a method for identifying an agent which affects the level/activity of, among others a molecule capable of hybridizing with the complement of SEQ ID NO:2. The subject-matter of claims 35-37 is novel and complies with the requirements of Article 33(2) PCT.

4.1. However, the attention of the applicant is drawn to the fact that document D1 discloses a molecule capable of hybridizing with the complement of SEQ ID NO:2. The identification of an agent affecting the level/activity of a known polypeptide is a routine manipulation in the state of the art, which the skilled person would realize without the need of exercising any inventive skill. Claim 35, consequently, lacks inventive step and does not meet the requirements of Article 33(3) PCT.

4.2. Claims 36 and 37 involve the above mentioned method specifying the function of the protein as protecting cells against degeneration and/or cell death. For the reasons already put forward in paragraph 1.2 above it is considered that claims 36 and 37 involve an inventive step and comply with the requirements of Article 33(3) PCT.

Re Item VI

Certain documents cited

Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*
29th Annual Meeting of the Society for Neuroscience, Miami Beach, Florida, USA Greeve et al.:"Expression of Seladin-1, a novel neuroprotective gene with homologies to oxido-reductases is associated with selective vulnerability in Alzheimer's disease."	23-28/10/1999	10/1999

Re Item VII

Certain defects in the international application

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 and D2 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII

Certain observations on the international application

1. The use of the term "isolated" in claims 1-7 introduces an unclarity according Article 6 PCT into the scope of the claims since the degree of isolation is not a technical feature of a product.

2. The use of the term "variant" in claims 2, 15, 18, 21, 24, 25, 35, 36 and 38 should be avoided, because it does not appear to have a precise meaning, thus rendering the scope of the claims unclear, cf. Article 6 PCT.

3. Claims 4, 13, 21, 22, 23 and 25 include, by means of the expression "in particular", optional features which do not have any limiting effect in the scope of the claims. The deletion of these features would improve the clarity of the claims as requested according to Article 6 PCT.

4. The drafting of claims 21-23, 35 and 38, including a plurality of options directed to very similar subject-matter renders these claims unclear. The different features (a) to (f) have been drafted as separate independent products, while they appear to relate effectively to overlapping subject-matter and to differ from each other only in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent options makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 21-23, 35 and 38 do not meet the requirements of Article 6 PCT.

WO 00/29569
PCT/EP99/08744

AvK | Sg | W | Da | Hi | HPJ | Me | TW | JH | KB

05. JUN 2000

K | F.12.05.ON/12.03.01

PATENT COOPERATION TREATY

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:
MEYERS, Hans-Wilhelm
P.O. Box 10 22 41
D-50462 Cologne
ALLEMAGNE

Date of mailing (day/month/year) 25 May 2000 (25.05.00)	
Applicant's or agent's file reference 992514wo Me/kk	**IMPORTANT NOTICE**
International application No. PCT/EP99/08744	**International filing date** (day/month/year) 12 November 1999 (12.11.99)
Priority date (day/month/year) 12 November 1998 (12.11.98)	
Applicant NITSCH, Roger et al	

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   EP

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   25 May 2000 (25.05.00) under No. WO 00/29569

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/308 (July 1996)

3293656

Continuation of Form PCT/IB/308

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

Date of mailing (day/month/year)	IMPORTANT NOTICE
25 May 2000 (25.05.00)	
Applicant's or agent's file reference	**International application No.**
992514wo Me/kk	PCT/EP99/08744

The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 46.1 for making amendments under Article 19 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.

AvK Sg W Da Hi HPJ ME TW JH KB

14. FEB. 2000

K F12 Oa. 00/12.05.04

PCT/EP99/08744

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

MEYERS, Hans-Wilhelm
P.O. Box 10 22 41
D-50462 Cologne
ALLEMAGNE

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

Date of mailing (day/month/year) 03 February 2000 (03.02.00)	
Applicant's or agent's file reference 992514wo Me/kk	**IMPORTANT NOTIFICATION**
International application No. PCT/EP99/08744	International filing date (day/month/year) 12 November 1999 (12.11.99)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 12 November 1998 (12.11.98)
Applicant NITSCH, Roger et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
12 Nove 1998 (12.11.98)	98121478.6	EP	17 Janu 2000 (17.01.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Taïeb Akremi

Telephone No. (41-22) 338.83.38

AwK | Sg | W | Da | Hi | HPJ | ME | TW | JH | KB

17. APR. 2000

K | Ka W

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

MEYERS, Hans-Wilhelm
P.O. Box 10 22 41
D-50462 Cologne
ALLEMAGNE

Date of mailing (day/month/year) 06 April 2000 (06.04.00)	
Applicant's or agent's file reference 992514wo Me/kk	**IMPORTANT NOTIFICATION**
International application No. PCT/EP99/08744	**International filing date** (day/month/year) 12 November 1999 (12.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
NITSCH, Roger Hartungstrasse 8 D-20416 Hamburg Germany	DE	DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
NITSCH, Roger Guggerstrasse 19 CH-8702 Zollikon Switzerland	DE	CH
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Dorothée Mülhausen
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 June 2000 (07.06.00)	
International application No. PCT/EP99/08744	**Applicant's or agent's file reference** 992514wo Me/kk
International filing date (day/month/year) 12 November 1999 (12.11.99)	**Priority date** (day/month/year) 12 November 1998 (12.11.98)
Applicant NITSCH, Roger et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

20 May 2000 (20.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. E. Stoffel
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

To:

MEYERS, Hans-Wilhelm
P.O. Box 10 22 41
D-50462 Cologne
ALLEMAGNE

AVK | Sg | W | Da | Hi | HPJ | ME | TW | JH | KB

07. FEB. 2000

K F 12.06.00 / 12.05.00

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
28 January 2000 (28.01.00)	
Applicant's or agent's file reference	**International application No.**
992514wo Me/kk	PCT/EP99/08744

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

NITSCH, Roger et al (all designated States)

International filing date	:	12 November 1999 (12.11.99)
Priority date(s) claimed	:	12 November 1998 (12.11.98)
Date of receipt of the record copy by the International Bureau	:	12 January 2000 (12.01.00)
List of designated Offices	:	

AP :GH,GM,KE,LS,MW,SD,SL,SZ,TZ,UG,ZW
EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
National :AE,AL,AU,BA,BB,BG,BR,CA,CN,CR,CU,CZ,DM,EE,GD,GE,HR,HU,ID,IL,IN,IS,JP,KP,
KR,LC,LK,LR,LT,LV,MA,MG,MK,MN,MX,NO,NZ,PL,RO,SG,SI,TR,TT,TZ,UA,US,UZ,VN,YU,ZA

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- [X] time limits for entry into the national phase
- [X] confirmation of precautionary designations
- [X] requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

P. Regis

Telephone No. (41-22) 338.83.38

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. **It is the applicant's responsibility** to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/12, C07K 14/47, C12Q 1/68, G01N 33/68, A61K 38/17

A1

(11) International Publication Number: WO 00/29569

(43) International Publication Date: 25 May 2000 (25.05.00)

(21) International Application Number: PCT/EP99/08744

(22) International Filing Date: 12 November 1999 (12.11.99)

(30) Priority Data:
98121478.6 12 November 1998 (12.11.98) EP

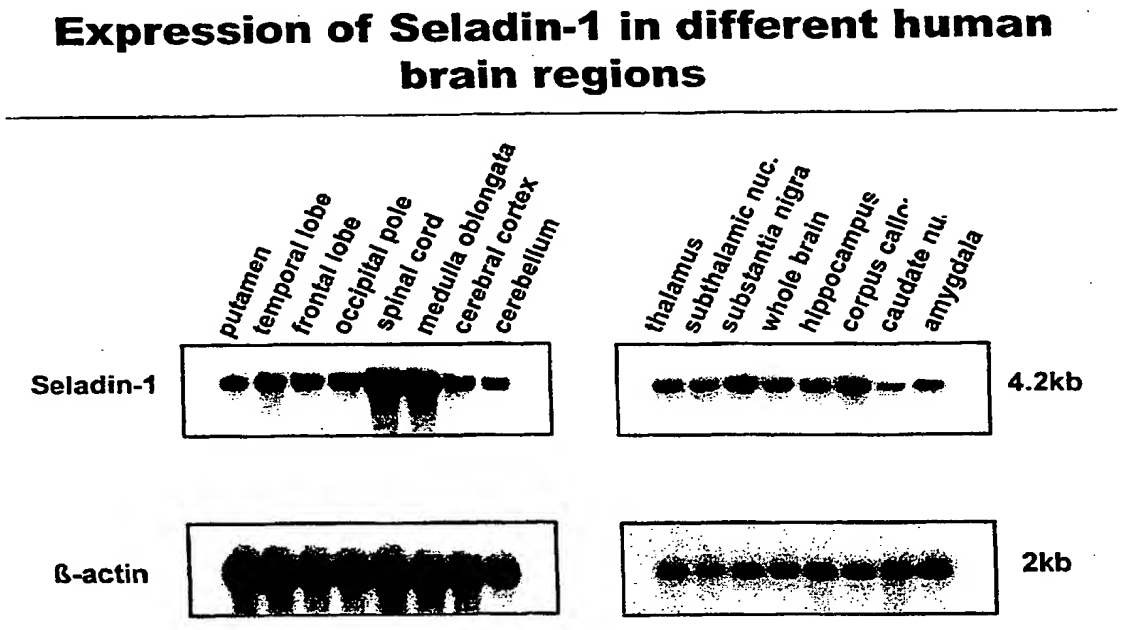
(71)(72) Applicants and Inventors: NITSCH, Roger [DE/CH]; Gugger Strasse 19, CH-8702 Zollikon (CH). GREEVE, Isabell [DE/DE]; Oberstrasse 108, D-20143 Hamburg (DE).

(74) Agents: MEYERS, Hans-Wilhelm et al.; P.O. Box 10 22 41, D-50462 Cologne (DE).

(81) Designated States: JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS OF DIAGNOSING OR TREATING NEUROLOGICAL DISEASES



(57) Abstract

The invention discloses an isolated nucleic acid molecule encoding a protein molecule, the function of which is to protect cells against degeneration and/or cell death, wherein the amino acid sequence of the protein comprises the sequence shown in SEQ ID NO. 2 or functional variant thereof.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

METHODS OF DIAGNOSING OR TREATING NEUROLOGICAL DISEASES

Cell death is a common feature occuring in two distinct forms in nature. Necrosis results from physical or chemical insult while apoptosis or programmed cell death results from a self-destruction program within the cell in response to internal and external stimuli. Latter process is a gene-directed form of cell death that is essential for normal development and maintenance of multicellular organisms. Recent work has clearly demonstrated that dysregulation of apoptosis may underlie the pathogenesis of a variety of diseases. Apoptosis has been reported to occur in conditions characterized by ischaemia, e.g. myocardial infarction and stroke. It has been implicated in a number of liver disorders including obstructive jaundice. Hepatic damage due to toxins and drugs is also associated with apoptosis in hepatocytes. Apoptosis has also been identified as a key phenomenon in some diseases of the kidney, i.e. polycistic kidney, as well as in disorders of the pancreas like alcohol-induced pancreatitis and diabetes. AIDS and neurodegenerative disorders like Alzheimer's and Parkinson's disease represent the most widely studied group of disorders where an excess of apoptosis has been implicated. Amyotrophic lateral sclerosis, retinitis pigmentosa, epilepsy and alcoholic brain damage are other neurological disorders in which apoptosis has been implicated.

Neurological diseases are widely spread within a population and have a strong impact not only on patients' life but also on society as such. Therefore, there is a strong need to elucidate the causes and the underlying pathogenesis of such neurological diseases. Among such neurological diseases, Alzheimer's disease (AD) has a predominant position. Alzheimer's disease, first described by the Bavarian psychiatrist Alois Alzheimer in 1907, is a progressive neurological disorder which begins with short term memory loss and proceeds to loss of cognitive functions, disorientation, impairment of judgement and reasoning and, ultimately, dementia. It is the most common cause of dementia. AD has been estimated to afflict 5 to 11 percent of the population over age 65 and as much as 47 percent of the population over age 85. Moreover, as adults, born during the population boom of the 1940's and 1950's, approach the age when AD becomes more prevalent, the control and treatment of AD will become an even more

significant health care problem. Familial forms of AD are genetically heterogeneous, but most with early onset are linked to mutations in the presenilin genes *PSEN1* and *PSEN2*, as well as to mutations of the amyloid precursor gene *APP*. The majority of AD patients have no obvious family history and are classified as sporadic AD. The neuropathology of AD is characterized by a substantial loss of neurons and synapses, and by the formation in brain of amyloid plaques and neurofibrillary tangles. Amyloid plaques are evenly distributed throughout the neocortex and the hippocampus, whereas neurodegeneration occurs predominantly in the inferior temporal lobes, the entorhinal cortex, and the hippocampus. Similar neurons in the frontal, parietal, and occipital lobes are largely preserved from degeneration even in severe end-stage AD. These observations indicate selective vulnerability of specific population of neurons. Factors that determine selective vulnerability of neurons in AD brains are unknown.

To elucidate the causes of cell degeneration and cell death is a general aim of the present invention. More specifically, the present invention aims at elucidating the causes and the underlying pathogenesis of neurological diseases, in particular Alzheimer's disease. It is therefore an object of the present invention to provide an insight into the pathogenesis of neurological diseases and to provide methods and materials which are suited for diagnosis and treatment of said diseases, cell degeneration and cell death.

The invention features an isolated nucleic acid molecule encoding a protein molecule whose amino acid sequence comprises the sequence shown in SEQ ID NO. 1 as well as the protein molecule according to SEQ ID NO.1. Hereinafter, the protein molecule of SEQ ID NO. 1 is denoted "SELADIN-1". One function of SELADIN-1 is to protect cells against degeneration and cell death. In particular, cells of the nerve system, muscular system, prostate, stomach, testis, ovary, adrenal glands, mammary glands, liver, spleen, lung, trachea or placenta are protected against degeneration and/or cell death. Therefore, the present invention also features functional variants of SELADIN-1 which might have a modification of the given primary structure of SELADIN-1, but whose essential biological function remains unaffected. "Variants" of a protein molecule shown in SEQ ID NO.1 include for example proteins with conservative amino acid substitutions

in highly conservative regions. For example, isoleucine, valine and leucine can each be substituted for one another. Aspartate and glutamate can be substituted for each other. Glutamine and asparagine can be substituted for each other. Serine and threonine can be substituted for each other. Amino acid substitutions in less conservative regions include e.g.: Isoleucine, valine and leucine can each be substituted for one another. Aspartate and glutamate can be substituted for each other. Glutamine and asparagine can be subsituted for each other. Serine and threonine can be substituted for each other. Glycine and alanine can be substituted for each other. Alanine and valine can be substituted for each other. Methionine can be substituted for each of leucine, isoleucine or valine, and vice versa. Lysine and arginine can be substituted for each other. One of aspartate and glutamate can be substituted for one of arginine or lysine, and vice versa. Histidine can be substituted for arginine or lysine, and vice versa. Glutamine and glutamate can be substituted for each other. Asparagine and aspartate can be substituted for each other. Other examples of protein modifications include glycosilation and further posttranslational modifications. The invention also features the nucleic acid molecules encoding such functional variants of the protein molecule of SEQ ID NO. 1. Nucleic acid molecules can be DNA molecules, such as genomic DNA molecules or cDNA molecules, or RNA molecules, such as mRNA molecules. In particular, said nucleic acid molecule can be a cDNA molecule comprising a nucleotide sequence of SEQ ID NO. 2. The invention also features an isolated D N A molecule capable of hybridizing with the complement of the cD N A described in SEQ ID NO. 2 under stringent conditions. Examples for stringent conditions include (i) 0.2xSSC (standard saline citrate) and 0.1 % SDS at 60 °C and (ii) 50 % formamide, 4xSSC, 50 mM HEPES, pH 7.0, 10x Denhardt's solution, 100 µg/ml thermally denatured salmon sperm DNA at 42 °C.

In another aspect, the invention features a vector comprising a nucleic acid encoding a protein molecule shown in SEQ ID NO. 1. It also features a vector comprising a nucleic acid molecule encoding a protein molecule, the function of which is to protect cells against degeneration and/or cell death, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO. 1 or a functional variant thereof. In preferred embodiments, a virus, a bacteriophage, or a plasmid comprises the

described nucleic acid. In particular, a plasmid adapted for expression in a bacterial cell comprises said nucleic acid molecule, e.g. a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, and the regulatory elements necessary for expression of said molecule in the bacterial cell. In a further aspect, the invention features a plasmid adapted for expression in a yeast cell which comprises said nucleic acid molecule, e.g. a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, and the regulatory elements necessary for expression of said molecule in the yeast cell. In another aspect, the invention features a plasmid adapted for expression in a mammalian cell which comprises a nucleic acid molecule, e.g. a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO.1, and the regulatory elements necessary for expression of said molecule in the mammalian cell.

In a further aspect, the invention features a cell comprising a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1. The invention also features cells comprising a nucleic acid molecule encoding a protein molecule whose function is to protect cells against degeneration and/or cell death and whose amino acid sequence comprises the sequence shown in SEQ ID NO. 1 or a functional variant thereof. It also features cells comprising a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions. In preferred embodiments, said cell is a bacterial cell, a yeast cell, a mammalian cell, or a cell of an insect. In particular, the invention features a bacterial cell comprising a plasmid adapted for expression in a bacterial cell, said plasmid comprising a nucleic acid molecule which encodes a protein molecule shown in SEQ ID NO. 1, and the regulatory elements necessary for expression of said molecule in the bacterial cell. The invention also features a yeast cell comprising a plasmid adapted for expression in a yeast cell, said plasmid comprises a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, and the regulatory elements necessary for expression of said molecule in the yeast cell. It further features a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, said plasmid comprising a nucleic acid molecule which encodes a protein molecule shown in SEQ ID NO.1, and the regulatory elements necessary for expression of said molecule in the mammalian cell.

The invention further features an antibody specifically immunoreactive with an immunogen, wherein said immunogen is shown in SEQ ID NO. 1 or wherein said immunogen is a protein molecule, the function of which is to protect cells against degeneration and/or cell death, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO. 1 or a functional variant thereof. In another aspect, the invention aims at a method of detecting pathological cells in a subject which comprises immunocytochemically staining cells with the aforementioned antibody, wherein a low degree of staining in said cell compared to a reference cell representing a known health status indicates a pathological change of said cell. The invention is particularly suited to detect pathological structures in the brain of a subject – the detection method comprises immunocytochemically staining said pathological structures with said antibody. It is also especially suited to detect pathological cells of the muscular system, prostate, stomach, testis, ovary, adrenal glands, mammary glands, liver, spleen, lung, trachea or placenta.

In another aspect, the invention features a method of diagnosing or prognosing a disease, in particular a neurological disease, in a subject comprising:
determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f),

and comparing said level, or said activity, or both said level and said activity, of at least one of said substances (a) to (h) to a reference value representing a known disease or health status, thereby diagnosing or prognosing a disease, in particular a neurological disease, in said subject.

In another aspect, the invention features a method of monitoring the progression of a disease, in particular a neurological disease, in a subject, comprising:

determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f),

and comparing said level, or said activity, or both said level and said activity, of at least one of said substances (a) to (h) to a reference value representing a known disease or health status, thereby monitoring progression of a disease, in particular a neurological disease, in said subject.

In still a further aspect, the invention features a method of evaluating a treatment for a disease, in particular a neurological disease, in a subject, said method comprising:

determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f),

and comparing said level, or said activity, or both said level and said activity, of at least one of said substances (a) to (h) to a reference value representing a known disease or health status, thereby evaluating a treatment for a disease, in particular a neurological disease, in said subject.

In a further aspect, the invention features a kit for diagnosis, or prognosis of a disease, said kit comprising:

(1) at least one reagent which is selected from the group consisting of reagents that selectively detect

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f),

(2) instructions for diagnosing, or prognosing said disease by

(i) detecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (h) in a sample from said subject;

and

(ii) diagnosing, or prognosing said disease, wherein

a varied level, or activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (h) compared to a reference value representing a known health status;

or a level, or activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (h) similar or equal to a reference value representing a known disease status indicates diagnosis, or prognosis of said disease.

In a further aspect, the kit may be used in monitoring success or failure of a therapeutic treatment of said subject. It can also be used in monitoring the progression of a disease.

Preferred embodiments of the above mentioned methods and kit of diagnosing or prognosing diseases, or monitoring the progression thereof, or evaluating a treatment thereof, are now disclosed in detail.

In a preferred embodiment, the function of said protein molecule or a functional variant thereof is to protect cells from degeneration and/or cell death.

In another preferred embodiment, said D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 encodes a protein molecule, the function of which is to protect cells against cell degeneration and/or cell death.

In preferred embodiments, said subjects suffer from Alzheimer's disease and related neurofibrillary disorders, or degenerative states, e.g. neurodegenerative states, characterized by cell degeneration or cell death. Further examples of neurological diseases are Parkinson´s disease, Huntington disease, amyotrophic lateralsclerosis and Pick´s disease.

It is particularly preferred that said sample is a brain tissue or other body cells including cells of the muscular system, prostate, stomach, testis, ovary, adrenal glands, mammary glands, liver, spleen, lung, trachea, or placenta. The sample might also be cerebrospinal fluid or another body fluid.

According to the present invention, a reduction in the level, or activity, or both said level and said activity, of (i) a transcription product of a D N A molecule encoding a protein molecule, whose amino acid sequence comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof or (ii) a protein molecule whose amino acid sequence comprises the sequence shown in SEQ ID NO. 1 or a functional variant thereof, in a sample from said subject relative to a reference value representing a known health status indicates the presence of a pathological status in said subject. In particular, a reduction in the level, or activity, or both said level and said activity of SELADIN-1 or *SELADIN-1* transcripts in said subject's brain regions affected heavily by neurodegeneration relative to a reference value representing a known health status indicates a diagnosis or prognosis of Alzheimer's disease. Predominantly neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus and the amygdala degenerate in Alzheimer's disease.

It might be preferred that said subject has previously been determined to have one or more factors indicating that such subject is afflicted with a disease under study, in particular a neurological disease.

In preferred embodiments, said subject can be a human, an experimental animal, e.g. a rat or a mouse, a domestic animal, or a non-human primate, e.g. a monkey. The experimental animal can be an animal model for a disorder, e.g. a transgenic mouse with an Alzheimer's-type neuropathology.

In preferred embodiments, at least one of said substances is detected using an immunoassay, an enzyme activity assay and/or a binding assay.

In preferred embodiments, measurement of the level of transcription products of the *SELADIN-1* gene, or a functional variant thereof, is performed in body cells using Northern blots with probes specific for the *SELADIN-1* gene or said variant. Quantitative PCR with primer combinations to amplify *SELADIN-1* gene-specific sequences from cDNA obtained by reverse transcription of RNA extracted from body cells of a subject can also be applied. These techniques are known to those of ordinary skill in the art (see e.g. Watson et al., Rekombinierte DNA, 2nd edition, Spektrum Akademischer Verlag GmbH, Heidelberg, 1993; Watson et al., Recombinant DNA, 2nd ed. W.H. Freeman and Company, 1992).

In preferred embodiments, said level or activity of the protein molecule shown in SEQ ID NO. 1, or a functional variant or fragment thereof, is detected using an immunoassay. These assays can measure the amount of binding between said protein molecule and an anti-protein antibody, e.g. an anti-SELADIN-1 antibody, by the use of enzymatic, chromodynamic, radioactive, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill

in the art (see Harlow et al., Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

The antibody or ligand to be used should preferably specifically detect SELADIN-1 or a functional variant or fragment thereof. It is preferred that it does not substantially interact with any other protein present in said sample.

Monoclonal antibodies capable of recognizing a protein molecule of SEQ ID NO. 1 or a functional variant or fragment thereof can be prepared using methods known in the art (see e.g. Köhler and Milstein, Nature 256, 495 - 497 1975; Kozbor et al., Immunol. Today 4, 72, 1983; Cole et al., Monoclonal antibodies and cancer therapy, Alan R. Liss, Inc., pp 77 - 96, 1985; Marks et al., J. Biol. Chem., 16007 - 16010, 1992; the contents of which are incorporated herein by reference). Such monoclonal antibodies or fragments thereof can also be produced by alternative methods known to those of skill in the art of recombinant DNA technology (see e.g. Sastry et al, PNAS 86: 5728, 1989; ; Watson et al., Rekombinierte DNA, 2nd ed., Spektrum Akademischer Verlag GmbH, 1993; Watson et al, Recombinant DNA, 2nd ed., W. H. Freeman and Company, 1992; the contents of which are incorporated herein by reference). Monoclonal antibodies useful in the methods of the invention are directed to an epitope of SELADIN-1 or a functional variant or fragment thereof, such that the complex formed between the antibody and SELADIN-1, or between the antibody and said functional variant or fragment, can be recognized in detection assays. The term "antibodies" encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, single chain antibodies as well as fragments thereof which specifically bind to SELADIN-1, or to a functional variant or fragment thereof.

Antibodies or ligands might also be used in detecting specifically molecules mentioned in the above described methods and kit under g) and h) above.

If luminescent labels are used in any detection assay, it is preferred to use a confocal optical set-up.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (h) described above in a sample from a subject not suffering of the disease under study, in particular a neurological disease such as Alzheimer's disease. The healthy subject can be of the same weight, age, and gender as the subject who is being diagnosed or prognosed for said disease. In some cases, it might be preferred to use a reference value from the subject which is diagnosed.

In a preferred embodiment, the level, or the activity, or both said level and said activity, of at least one of said substances (a) to (h) described above in a sample is determined at least twice, e.g. at two points which are weeks or months apart. The levels or activities at these two time points are compared in order to monitor the progression of said disease. It might be preferred to take a series of samples over a period of time. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings.

In another aspect, the invention features a method of treating or preventing a disease, in particular a neurological disease, in a subject comprising administering to said subject in a therapeutically effective amount an agent or agents which affect a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f).

In a preferred embodiment, the function of said protein molecule or a functional variant thereof is to protect cells from degeneration and/or cell death.

In another preferred embodiment, said D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 encodes a protein molecule, the function of which is to protect cells against cell degeneration and/or cell death.

In preferred embodiments, said subjects suffer from Alzheimer's disease and related neurofibrillary disorders, or degenerative states, such as neurodegenerative states, characterized by cell degeneration or cell death. Further examples of neurological diseases are Parkinson´s disease, Huntington disease, amyotrophic lateralsclerosis and Pick´s disease.

In preferred embodiments, the method comprises the application of per se known methods of gene therapy nucleic acid technology to administer said agent or said agents.

In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein,

and modulation of endogeneous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, Acc. Chem. Res. 26, 274 - 278, 1993; Mulligan, Science 260, 926 - 931, 1993; the contents of which are incorporated herein by reference) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane perturbation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, Current Opinion in Neurobiology, 3, 743 - 748, 1993; the contents of which are incorporated herein by reference).

In preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, said subject or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phospate transfection, liposomal mediated transfection, etc.

In preferred embodiments, said agent is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector.

In preferred embodiments, the therapeutic nucleic acid or protein reduces or prevents the degeneration of cells, in particular neurons and slows brain amyloid formation.

In another aspect, the invention features an agent which affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f).

Preferably, the function of said protein molecule or a variant thereof is to protect cells from degeneration and/or cell death. Preferably, said D N A molecule capable of

hybridizing with the complement of the c D N A described in SEQ ID NO. 2 encodes a protein, whose function is to protect cells from degeneration and/or cell death.

In another aspect, the invention features a medicament comprising such an agent.

In still another aspect, the invention features an agent for treating or preventing a disease, in particular a neurological disease, which agent affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f).

In preferred embodiments, said diseases are degenerative states characterized by cell degeneration or cell death or Alzheimer's disease and related neurofibrillary disorders. Further examples of neurological diseases are Parkinson's disease, Huntington disease, Amyotrophic lateralsclerosis, Pick's disease.

Preferably, the function of said protein molecule or a variant thereof is to protect cells from degeneration and/or cell death. Preferably, said D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 encodes a protein, whose function is to protect cells from degeneration and/or cell death.

In a further aspect, the invention features the use of an agent, for preparation of a medicament for treating or preventing a neurological disease, which agent affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a translation product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(h) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f).

Preferably, the function of said protein molecule or a variant thereof is to protect cells from degeneration and/or cell death. Preferably, said D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO.2 encodes a protein molecule, whose function is to protect cells against degeneration and/or cell death.

In preferred embodiments, said diseases are Alzheimer's disease and related neurofibrillary disorders, or degenerative states, in particular neurodegenerative states, characterized by cell degeneration or cell death. Further examples of neurological diseases are Parkinson's disease, Huntington disease, Amyotrophic lateralsclerosis, Pick's disease.

In a further aspect, the invention features a method for identifying an agent that affects an activity, or level, or both said activity or level, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f),

comprising the steps of:

(i) providing a sample containing at least one substance which is selected from the group consisting of (a) to (f),

(ii) contacting said sample with at least one agent,

(iii) comparing an activity, or level, or both said activity and level, of at least one of said substances before and after contacting.

Preferably, the function of said protein molecule or a variant thereof is to protect cells from degeneration and/or cell death. Preferably, said D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 encodes a protein molecule, whose function is to protect cells against degeneration and/or cell death.

Other features and advantages of the invention will be apparent from the following detailed description of the figures, the examples and the claims.

Figure 1 depicts the selective vulnerability of brain regions in Alzheimer's disease. Predominantly neurons within the inferior temporal lobe, the entorhinal cortex, the

hippocampus and the amygdala degenerate in Alzheimer´s disease (Terry et al., Annals of Neurology, 10, 184-192, 1981). These brain regions are predominantly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex and the cerebellum are largely intact and preserved from the neurodegenerative process in Alzheimer´s disease.

Figure 2 discloses the identification of genes differentially expressed in brain regions from Alzheimer´s disease patients. Brain areas with massive neuronal cell loss as well as areas with largely preserved neurons were identified and RNA extracted. Synthesis of cDNA was performed using an oligo-dT primer followed by PCR using the oligo-dT primer in combination with random primers and (α^{35}S)-dATP. Reactions were separated on DNA sequencing gels, DNA bands visualized by autoradiography and bands lighting up in different intensities were cut out. DNA fragments were reamplified by PCR, cloned in *E. coli* and sequences determined. Expression and functional analyses were performed.

Figure 3 depicts the specifications of Alzheimer´s disease brain tissue as it was used in the examples. Brain tissues from Alzheimer´s disease patients and control subjects were removed within 6 hours of death, and immediately frozen on dry ice. For RNA extraction tissue sections from the inferior temporal lobe and frontal cortex were chosen.

Figure 4 discloses the quantification of *SELADIN-1* transcripts in brain tissue from Alzheimer´s disease and control subjects by Northern blot analyses. Transcript levels were significantly lower in brain regions with severe neurodegeneration, i. e. temporal lobe in Alzheimer´s disease (AD1-3) but not in normal brain (NB1-3), as compared to protected brain regions, i. e. frontal lobe. This decrease was specific as indicated by unchanged ß-actin transcript levels used to control for equal loading of RNA.

Figure 5 depicts the transcription levels of the *SELADIN-1* gene in different human brain regions. The *SELADIN-1* gene was found to be expressed throughout the human brain. In particular transcription levels are high in all cortical areas, the hippocampus, the

amygdala, the spinal cord, and the medulla. Note the unchanged levels in temporal lobe versus frontal lobe in this brain derived from a cognitively normal control subject without any signs of Alzheimer's disease. The analysis of ß-actin transcripts was used as loading control.

Figure 6 depicts the distribution of *SELADIN-1* transcripts in human tissues. Comparable samples of RNA were spotted on nitrocellulose filters and *SELADIN-1* transcripts were quantified by hybridization using a labeled SELADIN-1 gene specific probe. Significant levels of *SELADIN-1* gene transcripts were found in all brain regions tested. Transcripts were also detected in other tissues, however, strong variations in signal intensity indicated a tissue specific regulation of *SELADIN-1* expression.

Figure 7 depicts the expression of the *SELADIN-1* gene in rat brain cortex, hippocampus and basal nucleus analyzed by in situ hybridization. This staining pattern along with the higher magnifications indicate that *SELADIN-1* is predominantly expressed in neurons. No significant hybridization signals were observed with glial cells.

Figure 8 depicts the expression of *SELADIN-1* in rat brain nuclei. Strong *SELADIN-1* expression was found in the occulomotor, paraventricular, red and facial nuclei. Higer magnifications indicate predominant hybridization with neurons. No significant hybridization signals were observed with glial cells.

Figure 9 depicts the expression of *SELADIN-1* in rat brain hippocampus and substantia nigra. In situ hybridization with *SELADIN-1* transcripts was detected by photoemulsionautoradiography, confirming the neuron specific expression of this gene.

Figure 10 discloses the subcellular localization of a SELADIN-1-EGFP (enhanced green fluorescent protein) fusion in transfected cos cells. The confocal micrographs show the co-localization of the SELADIN-1-EGFP fusion with the golgi specific stain BODIPY TR ceramide indicating localization of SELADIN-1 in the Golgi apparatus and the endoplasmic reticulum.

Figure 11 discloses that the SELADIN-1-EGFP fusion does not localize to mitochondria in transfected cos cells in spite of a putative mitochondrial targeting sequence close to the N-terminus of the SELADIN-1 protein. The confocal micrographs show the different staining patterns caused by the SELADIN-1-EGFP fusion and the specific mitochondrial stain Mito Tracker Red CM-H_2XRos.

Figure 12 discloses structural features of the SELADIN-1 protein based on multiple sequence alignments and secondary structure predictions. Near the N-terminus the SELADIN-1 protein contains a putative mitochondrial localization signal that appears to be inactive in transfected cos cells or when used in EGFP fusions. The central region of the protein contains a sequence that is homologous to a family of oxidoreductases and that contains a FAD site for covalent binding. The protein is predicted to contain five transmembrane regions. The expression in neurons, the co-localization in the Golgi apparatus and the endoplasmic reticulum of the SELADIN-1 protein, the amyloid precursor protein (APP) and the presenilins PS1 and PS2 and furthermore the transmembrane character suggest a functional relationship between these proteins. Mutations in both APP and presenilins were shown to cause an increase in the production of ß-amyloid. In a similar way the SELADIN-1 protein might be involved in common biological pathways influencing the processing of the amyloid precursor protein and the generation of Aß. Using the SELADIN-1 protein as a probe, interaction partners can be identified which might represent new AD drug targets.

Figure 13 discloses the protein sequence of SELADIN-1 (SEQ ID NO. 1). The full length protein consists of 516 amino acid residues. The sequence is given in the one letter amino acid code.

Figure 14 discloses the nucleotide sequence of the cloned *SELADIN-1* cDNA (SEQ ID NO. 2) comprising 4248 nucleotides. The coding sequence for the SELADIN-1 protein starts at nucleotide position 100 and stops at position 1648.

Figure 15 discloses the comparison of nucleotide sequences of the cloned *SELADIN-1* cDNA comprising 4248 nucleotides and the KIAA0018 cDNA comprising 4186

nucleotides. A significant difference exists at position 1228 of the *SELADIN-1* sequence where a C nucleotide (C/G basepair) is missing in the *KIAA0018* sequence. This results in a frameshift in the open reading frame in the *KIAA0018* sequence relative to the *SELADIN-1* sequence. The consequence is that the translation product of the *KIAA0018* gene is 390 amino acids in length compared to 516 amino acid residues of the SELADIN-1 translation product. In addition to the difference in length, the frameshift causes a difference between the C-terminal 14 amino acids of the KIAA0018 protein and the corresponding sequence area of the SELADIN-1 polypeptide (pos. 377 - 390). The coding sequence for the SELADIN-1 protein starts at nucleotide position 100 and stops at position 1648.

Figure 16 shows the amino acid sequence of SELADIN-1. A differential display approach (von der Kammer, H. et al., Nucleic acid research, 27, 2211, 1999; von der Kammer, H. et al., J. Biol. Chem. 273, 14538, 1998) to identify genes that are differentially expressed in selectively vulnerable cell populations in the inferior temproal cortex with confirmed neurodegeneration and in the largely unaffected frontal or sensory-motor cortex of the same subject in three brains with a histopathological diagnosis of Alzheimer's disease and post mortem time intervlas of less than four hours. By using forty different primer combinations, twenty-eight of thirty-six differentially expressed cDNAs were cloned and sequenced. These cDNAs were further analyzed by reverse Northern blotting (Poirier G.M.-C. et al., Nucleic Acid Res., 25, 913, 1997; Van Gelder R. N. et al., Proc. Natl. Acad. Sci. USA, 87, 1663, 1990) to confirm differential expression between the two AD brain regions. Expression of one of these cDNAs was markedly lower in the inferior temporal lobe than in the sensory-motor cortex. Therefore, the potential importance of this transcript for the selective vulnerability in AD brain has been investigated. The cDNA sequence consisted of 4248 nucleotides and encoded an open reading frame of 516 amino acid residues. Due to a cytidine insertion at nucleotide position 1167, this sequence differed from the much shorter coding region of its homolog KIAA0018 deposited in GenBank (Nomura et al., D N A Res. 1, 27, 1994; GenBank database accession HUMRSC390D13643,1, 1992; DIMH Human Q15392, 1998). The new gene has been designated *SELADIN-1*. The homology domain to oxido-reductases are highlighted in red; the homologies to "diminuto like proteins" of

other species are underlined. The first 21 amino acid residues represent a putative signal peptide. One possible caspase recognition motif is highlighted in yellow. This putative caspase recognition motif "LEVD" is present within the SELADIN-1 amino acid sequence at position 121 – 125. *In vitro* cleavage of SELADIN-1 by caspase 3 or 6 generated four different SELADIN-1 fragments of approximately 50, 40, 30 and 20 kDa, respectively. Secondary structure predictions revealed at least four possible transmembrane domains.

Figure 17 shows Northern blots of Alzheimer's disease (AD) brain and normal control brain. In AD brains, the expression of *SELADIN-1* was substantially lower in the inferior temporal lobe compared to the frontal cortex. In contrast, there was no difference in expression between these two regions in normal control brains (Fig. 17 A, B). Thus, the differential expression of *SELADIN-1* between temporal and frontal cortex within individual AD brains initially observed by both differential display and reverse Northerns, was independently confirmed in three other patients. *SELADIN-1* is strongly expressed throughout the normal human brain with highest expression in the cortices, in the medulla oblongata and the spinal cord as well as in substantia nigra and the hippocampus (Fig. 17B). **A** 10 µg of total RNA per lane, extracted with Trizol Reagent (Gibco) from the frontal cortex or the inferior temporal cortex of three different AD brains were separated on a 0.8 % formaldehyde-agarose gel and blotted on a Hybond-N+-Nylon Membrane (Amersham). Brain 1: post mortem time interval 3:30 hours, male, 72 years. Brain 2: post mortem time interval 1:30 hours, male, 62 years. Brain 3: post mortem time interval 4 hours, female, 63 years. Control brain: normal brain, post mortem time interval 1:10 hours, female, 80 years. The blots were hybridized with a ^{32}P-labeled c D N A probe of *Seladin-1* from nucleotide 1 – 3505 and with a ^{32}P-labeled c D N A control probe of human β-actin as provided by Clontech for the human brain multiple tissue northern blot II and III. **B** Human brain multiple tissue Northern blot II (Clontech 7755-1) and III (Clontech 7750-1) containing 2 µg of polyA+ RNA per lane from 16 different human brain regions. Blots were hybridized with the same probes as described in A.

Figure 18 shows the expression of *Seladin-1* in rat brain. *In situ* hybridization on paraformaldehyde fixed cryostat sections was performed as described by Hartman et al. (Developmental Neuroscience 17, 246, 1995). A 650 bp and a 900 bp fragment of the open reading frame of *Seladin-1* were PCR amplified using the following primer pairs:
1s (76-99) 5' GCG CTT ACC GCG CGG CGC CGC ACC 3' (SEQ ID NO. 3)
1as (749-726) 5' GAC CAG GGT ACG GCA TAG AAC AGG 3' (SEQ ID NO. 4)
3s (803-826) 5' AGA AGT ACG TCA AGC TGC GTT TCG 3' (SEQ ID NO. 5) and
3as (1749-1726) 5' TTC TCT TTG AAA GTG TGG ATC TAG 3' (SEQ ID NO. 6).
PCR fragments were cloned in pGEM-Teasy vector (Promega), cut with EcoRI and cloned in pBluescript KS+. The orientation of the EcoRI cloned fragments was analyzed by PCR. Using the Ambion Maxiscript kit, ^{35}S-UTP labeled antisense and sense riboprobes were generated on NotI and ClaI linearized plasmids with T3 and T7-Polymerase, respectively, according to the manufacturers instructions. Hybridized sections were dipped in NTB-3 photographic emulsion (Kodak), exposed for 5 weeks and counterstained in Mayer's hemalum. **A, D, G** show photomicrographs of the emulsion dipped sections. **pvn** paraventricular nucleus, **bnM** basal nucleus of Meynert, **amy** amygdala, **ocmn** oculomotor nucleus, **rn** red nucleus, **fn** facial nucleus. **B** is a darkfield illumination blow up of the hippocampal region. **dg** dentate gyrus. **C** is a darkfield illumination blow up of the cortical layer five **cl V. E, H** show brightfield higher magnification photomicrographes of the regions of interest from D and G. **F, I** DIC (differential interference contrast) illuminations in higher magnification of E and H to demonstrate single neurons stained with silver grains. In rat brain, expression of *SELADIN-1* was high in the hippocampal region CA3 (Fig. 18 A, B), in the pyramidal neurons of cortical layer five (Fig. 18 A, C), in the amygdala (Fig. 18 A), in the magnocellular neurons of the basal nucleus of Meynert (Fig. 18 A) and in the reticular zone of the substantia nigra (data not shown). In addition, transcripts were also detected in several brain nuclei including the paraventricular nucleus (Fig. 18 A), the oculomotor nucleus (Fig. 18 D, E), the facial nucleus (Fig. 18 G, F) as well as the red nucleus (Fig. 18 D, E).

Figure 19 shows *in situ* hybridization of human AD (**A-D**) and normal brain (**E-H**). *In situ* hybridization on embedded sections was performed as described (U. Süsens, Dev. Neurosci. 19, 410, 1997). The ^{35}S-UTP labeled riboprobe was derived from the first 650 nucleotides of the open reading frame of *Seladin-1* cloned in pBluescript KS+ as described in Figure 18. The hybridized slides were dipped in Kodak NTB-2 emulsion, exposed for 4 weeks. After development, sections were stained with Giemsa. **A, C, E and G** show darkfield illuminations and **B, D, F, H** the corresponding brightfield photomicrographes. To enhance the visibility of the silver grains in the brightfield picture higher magnification is shown. **A, B** representative hybridization pattern of *Seladin-1* in midfrontal cortex of AD brain. **C, D** representative hybridization pattern of *Seladin-1* in superior temporal cortex of AD brain. **E, F** representative hybridization pattern of *Seladin-1* in midfrontal cortex of normal brain. **G, H** representative hybridization pattern of *Seladin-1* in superior temporal cortex of normal brain. Arrowheads indicate neurons packed with silver grains; arrows indicate the neurons with only few grains (D). *In situ* hybridization of human AD and control brains to study the expression of *SELADIN-1* within single neurons, demonstrated that *SELADIN-1* mRNA was reduced in the remaining neurons of the temporal cortex in comparison to the neurons in the frontal cortex in the AD brains (Fig. 19, A-D, arrows). In contrast, in normal brains, neuronal expression of *SELADIN-1* was identical between the frontal cortex and the temporal cortex (Fig. 19, E-H, arrowheads), confirming the data from differential display and Northern blot analyses. Reduced levels of *SELADIN-1* mRNA in the temporal cortex in comparison to the frontal cortex in the AD brain were not only due to cell loss but were also reduced within the remaining neurons.

Figure 20. To analyze SELADIN-1 function as a putative oxido-reductase, human H4 neuroglioma cells were stably transfected with Seladin-1 fused at its C-terminus to EGFP (enhanced green fluorescence protein, Clontech). **A** 10 and 16 hours after incubation of three seladin-1-EGFP clones and three EGFP-control clones in OptiMEM1 containing 200 µM H_2O_2, cells remaining attached to the culture dish as well as cells in the supernatant were harvested and stained with 7-Amino-actinomycin D (7-ADD) as a standard flow cytometric viability probe to distinguish viable from non viable cells. Only

membranes of dead and damaged cells are permeable to this D N A dye and stain positive. Live/dead counts were done on FACSCalibur (Becton Dickinson) counting 10^5 cells per clone. Means of 2 experiments in triplicate are shown (± SEM). All SELADIN-1 expressing clones tolerated H_2O_2-induced oxidative stress much better than either non-transfected or EGFP expressing clones. After ten hours treatment with 200 µM H_2O_2 nearly 90 % of the SELADIN-1 expressing cells and 75 – 80 % of the control cells were viable; sixteen hours after incubation with 200 µM H_2O_2, however, 80 % of the SELADIN-1 expressing cells were still alive whereas only 52 % of the control cells were alive at this time point. Untreated control clones revealed a maximum of 5 % dead cells at equivalent time intervals. Increased survival rates in SELADIN-1 expressing cells after prolonged exposure to oxidative stress was confirmed by two independent approaches: First, live/dead counts were done on trypan blue stained cells on cell culture dishes and visualized in phase-contrast microscopy in ten randomly chosen fields. Second, nuclei of cells grown on coverslips and fixed with 4 % paraformaldehyde were stained with Hoechst dye 33342 (Molecular Probes) and visualized by fluorescence microscopy (data not shown). These measures confirmed that expression of SELADIN-1 conferred resistance against induction of cell death.

B To determine an early marker for apoptotic cell death, the activity of caspase 3 in cell lysates of three SELADIN-1-EGFP clones and three EGFP-control clones was measured using the caspase 3 assay kit from Pharmingen. After induction of apoptosis with 200 µM H_2O_2 for 2 or 4 hours, respectively, cells were washed briefly in PBS and lysed in 10 mM Tris-HCl, pH 7.5, 10 mM NaH_2PO_4, pH 7.5, 130 mM NaCl, 1 % Triton-X-100, 10 nM NaPPi (2 million cells/ml). 50 µl of the cell lysates were incubated in 200 µl HEPES buffer for 1 hour at 37 °C with 5 µg of the caspase 3 fluorogenic substrate Ac-DEBD-CHO in a 96 multiwell plate. The AMC liberated from Ac-DEVD after caspase cleavage was measured on a spectrofluorometer (Spectramax Gemini, Molecular Devices) with an excitation wavelength of 380 nm and an emission wavelength spectrum from 420 – 460 nm. Means of caspase 3 activity, measured in RFU (relative fluorescence units) of two experiments in triplicates are shown (± SEM). Two hours after induction of apoptosis with 200 µM H_2O_2, caspase 3 activity was not detectable in either SELADIN-1-EGFP clones or in the EGFP-control clones. After 4 hours, however, the activity of caspase 3 strongly increased and was found to be approximately two-fold

higher in three EGFP-control clones as compared to three SELADIN-1-EGFP clones. This increase in caspase 3 activity was blocked in either condition by the caspase inhibitor Ac-DEVD-CHO.

Figure 21 shows the subcellular localization of SELADIN-1. 114 human neuroglioma cells that stable express a fusionprotein of SELADIN-1 with the N-terminus of EGFP (Clontech) were grown on coverslips and fixed in 4 % paraformaldehyde in PBS or treated for 45 minutes with 250 nM of the red fluorescent mitochondrial stain MitoTracker red CM H_2Xros (Molecular Probes) before fixation. After fixation cells that have not been prestained with the MitoTracker were permeabilized in 0.2 % Triton-X 100 in PBS and blocked over night at 4 °C in 5 % low fat milk, 0.1 % Triton-X 100 in PBS. Cells were incubated for 2 hours at room temperature with an monoclonal antibody against the mouse anti-protein disulfide isomerase (antiPDImAb, StressGen Biotechnologies Corp.), a marker for the endoplasmic reticulum, washed and incubated for another hour with an anti-mouse IgG, CY3 labeled secondary antibody (Amersham). Cells were visualized with confocal laser scanning microscopy. **A, D** Subcellular distribution of the green fluorescent SELADIN-1-EGFP fusionprotein. **B** Staining of the endoplasmatic reticulum with the antiPDImAb and the red fluorescent CY3 labeled secondary antibody. **C** Overlay from A and B shows the colocalization of SELADIN-1 with the ER-marker, indicated as yellow fluorescence. **E** Staining of the mitochondria with the red fluorescence MitoTracker CM H_2Xros. **F** Overlay of D and E. These colocalization studies with markers and antibodies against several subcellular organelles indicated that SELADIN-1-EGFP mainly localized to the endoplasmatic reticulum and not to the mitochondria, despote the presence of a putative mitochondrial localization signal at the N-terminus of SELADIN-1.

Taken together a novel gene *SELADIN-1* that has homologies to FAD-dependent oxido-reductases has been identified. It has been shown that it was down-regulated in selectively vulnerable regions of AD brain. *In situ* hybridization of AD brain sections demonstrated that the reduced mRNA levels are not only due to neuronal loss in affected areas but also reflects reduced mRNA expression of the remaining neurons. Expression of *SELADIN-1* in H4 cells conferred resistance to apoptosis by oxidative

stress, yet after execution of apoptosis SELADIN-1 is cleaved at putative caspase cleavage sites and therefore is presumably inactivated. These results indicate that SELADIN-1 is an integral component of the cellular machinery protecting cells, in particular neurons, from oxidative stress. Once oxidative stress becomes overwhelming, SELADIN-1 becomes a target for caspase action in the course of apoptosis. *SELADIN-1* is a good candidate gene for therapeutical intervention to protect cells against degeneration and cell death. It is in particular, a good candidate gene for therapeutical intervention to protect neurons from Aβ induced cytotoxicity.

EXAMPLE I

Post-mortem Alzheimer's disease brain tissues

Brain tissues from Alzheimer's disease patients and control subjects were removed within 6 hours of death, and immediately frozen on dry ice. Parallel sections were fixed in formaldehyde for histopathological confirmation of the diagnosis and for cell counts. Brain areas with massive neuronal cell loss as well as areas with largely preserved neurons were identified for comparisons of gene expression and stored at -80°C until RNA extractions were performed.

Identification of *SELADIN-1* by differential display PCR

Total RNA from post-mortem brain tissues was prepared by using the RNeasy kit (Qiagen). The RNA preparations were treated with DNase I (Boehringer Mannheim) together with RNAsin (Promega) for 30 minutes, followed by phenol extraction, and ethanol precipitation. 0.2 mg of each RNA preparation were transcribed to cDNA by using Expand Reverse Transcriptase (Boehringer Mannheim) with one base ancor primers $HT_{11}A$, $HT_{11}C$ and $HT_{11}G$. In the following PCR reaction, the cDNAs were amplified by using $HT_{11}A$ along with the random primers HAP-5 (5'-TGCCGAAGCTTGGAGCTT-3') and HAP3-T (5'TGCCGAAGCTTTGGTCAT-3'). Taq-polymerase (AmpliTaq, Perkin Elmer Corp.), dGTP, dCTP, and dTTP (Amersham Pharmacia Biotech) and (α^{35}S)-dATP (NEN life science products) were used in a PCR protocol according to Zhao et al. The PCR products were separated on 6% polyacrylamide-urea sequencing gels that were dried subsequently on 3 mm filter paper (Whatman), and X-ray films (Dupont) were exposed for 12 hours.

Cloning and sequencing

Differential bands were excised from the gel, boiled in water for 10 minutes, centrifuged, and cDNAs were precipitated from the supernatant fluids by using ethanol and glycogen/sodiumacetate, followed by dialysis against 10% glycerol for 1 hour through 0.025 mm filters (type VS, Millipore). The dialysates were used as templates for the reamplification reactions that were done under identical conditions as in the differential

display PCR, with the exception of the initial cycle for nonspecific annealing. The resulting PCR products were separated by agarose gelelectrophoresis, purified from the gel with the QIAEXII Agarose Gel Extraction Kit (Qiagen), and cloned into the *Hind* III restriction site of pBluescript KS (Stratagene). Cloned cDNA fragments were sequenced with an ABI 377 DNA sequencer (Perkin Elmer Corp.) by using T3 and T7 primers.

Amplification of a *SELADIN-1* cDNA-fragment

A *SELADIN-1* cDNA fragment was amplified by using cDNA transcribed from human brain tissue by using RNA High Fidelity Taq-polymerase (Boehringer Mannheim) and *SELADIN-1*-specific primers for a PCR reaction with 40 cycles of annealing of 70 °C for 1 minute, and polymerization at 72 °C for 3 minutes. The PCR products were separated by agarose gel electrophoresis, purified, and cloned into the *Sma* I restriction site of pBluescript KS (Stratagene). The cloned PCR product was sequenced, and restriction were used as a probe both for screening a human brain cDNA library and for probing Northern blots.

Northern blotting

Total RNA from post-mortem human brains were prepared by using the Trizol reagent (Gibco BRL, Life Technologies), following the manufacturer's instructions. 5 - 10 mg of RNA were separated in 1 % formaldehyde-containing agarose gels, and the RNA was blotted onto nylon membranes (Hybond-N^+, Amersham). Membranes were hybridized with (α-^{32}P)-dCTP (NEN) labeled *SELADIN-1*-specific cDNA probes that were generated by using the Megaprime DNA labelling kit (Amersham). Membranes were washed under high stringency conditions, and X-ray films were exposed for 1 to 72 hours. To control for equal loading of RNA, the identical membranes were probed with a 700 pb cDNA fragment of human glycerolaldehyd-3-phosphate dehydrogenase (*GAPDH*), or with a b-actin cDNA fragment (Clontech).

In situ hybridization

Several *SELADIN-1*-specific cDNA probes of 650bp and of 900bp representing the initial two parts of the open reading frame were cloned in pBluescript (Stratagene) and reversely transcribed in the presence of ^{35}S-CTP by using the Ambion transcription kit. In situ hybridization was done with, 14mm sections of adult rat brain cut on a cryomicrotome, mounted on aminoalkylsilane-treated slides and fixed in 4 % paraformaldehyde in PBS for 5 min at room temperature. After washing for 5 min in PBS, sections were acetylated for 10 min, passed through a series of increasing ethanol grades and air dried. Prehybridizations were done in 50 % deionized formamide, 25 mM EDTA, 25 mM Pipes, pH 6.8, 0.75 M NaCl, 0.2 % SDS, 5 x Denhardt's, 10 mM DTT, 250 mg/ml denatured herring sperm DNA and 250 mg/ml yeast tRNA. Hybridization of slides with RNA sense and antisense probes diluted to 2000 – 5000 cpm/ml in the same buffer with additional 10 % dextransulphate was performed at 50 °C for 12 hours. Slides were then washed four times in 4 x SCC for 5 min. each, followed by an incubation for 30 min. at 37 °C with 40 mg/ml RNAseA in 0.5 M NaCl, 10 mM Tris-HCL, pH 7.5, 1mM EDTA and another 30 min without RNAseA. Then slides were washed twice for 15 min. at 50 °C in 2 x SCC and dried through graded ethanols. Slides were exposed to Kodak Biomax x-ray films for 15 days and subsequently dipped in Kodak NTB-3 nuclear track emulsion and exposed for 6 weeks. After developing in Kodak D19 and fixing in Kodak Unifix, slides were counterstained with Mayer" Hemalaun and coversplipped.

Recombinant expression of SELADIN-1-EGFP fusion proteins in tissue culture

The complete coding region of *SELADIN-1* was subcloned into the N-terminus of the pEGFP-N1-expression vector (Clontech). Cos-7-cells were transfected with *EGFP* or with *SELADIN-1-EGFP* by using the SuperFect transfection reagent from Qiagen according to the manufacturers instructions. Cells were cultured in 3 cm dishes for two days. Part of the cells were stained for the Golgi-apparatus with 0.25 mM BODIPY TR ceramide (molecular probes) for one hour, the other part was treated with 250 nM of the mitochondrial stain Mito Tracker Red CM-H2Xros (Molecular probes) for 45 min. the subcellular localization of the SELADIN-1-EGFP fusion protein was analyzed by confocal laser scanning microscopy using the appropriate filter sets.

CLAIMS

1. An isolated nucleic acid encoding a protein molecule shown in SEQ ID NO. 1.

2. An isolated nucleic acid molecule encoding a protein molecule, the function of which is to protect cells against degeneration and/or cell death, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof.

3. An isolated nucleic acid molecule of claim 1 or 2, wherein the nucleic acid molecule is a D N A molecule.

4. An isolated nucleic acid molecule of claim 3, wherein the nucleic acid molecule is a cD N A molecule, in particular a cD N A molecule comprising a nucleotide sequence shown in SEQ ID NO. 2.

5. An isolated D N A molecule capable of hybridizing with the complement of the cD N A described in SEQ ID NO. 2 under stringent condition.

6. An isolated D N A molecule of claim 5 encoding a protein molecule, the function of which is to protect cells against degeneration and/or cell death.

7. An isolated nucleic acid molecule of claim 2 or 5 encoding a protein molecule, the function of which is to protect cells of the nerve system, muscular system, prostate, stomach, testis, ovary, adrenal glands, mammary glands, liver, spleen, lung, trachea or placenta against degeneration and/or cell death.

8. A vector comprising a nucleic acid molecule according to one of claims 1 to 7.

9. A vector according to claim 8 wherein said vector is a plasmid, a virus or a bacteriophage.

10. A plasmid according to claim 9 wherein said plasmid is adapted for expression in a yeast cell and further comprises the regulatory elements necessary for expression of said nucleic acid molecule.

11. A plasmid according to claim 9 wherein said plasmid is adapted for expression in a bacterial cell and further comprises the regulatory elements necessary for expression of said nucleic acid molecule.

12. A plasmid according to claim 8 wherein said plasmid is adapted for expression in a mammalian cell and further comprises the regulatory elements necessary for expression of said nucleic acid molecule.

13. A cell transformed with a nucleic acid molecule according to one of claims 1 to 7, wherein said cell is in particular a bacterial cell, a yeast cell, a mammalian cell, or an insect cell.

14. A protein molecule shown in SEQ ID NO.1.

15. A protein molecule, the function of which is to protect cells against degeneration and/or cell death, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof.

16. A protein molecule of claim 14, the function of which is to protect cells of the nerve system, muscular system, prostate, stomach, testis, ovary, adrenal glands, mammary glands, liver, spleen, against degeneration and/or cell death.

17. An antibody specifically immunoreactive with an immunogen, wherein said immunogen is a protein molecule shown in SEQ ID NO. 1.

18. An antibody specifically immunoreactive with a protein molecule, the function of which is to protect cells against degeneration and/or cell death, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO. 1 or a functional variant thereof.

19. A method of detecting pathological cells in a subject which comprises immunocytochemically staining cells with an antibody of claim 17 or 18, wherein a low degree of staining in said cell compared to a cell representing a known health status indicates a pathological change of said cells.

20. A method of claim 19, wherein cells of the nerve system, muscular system, prostate, stomach, testis, ovary, adrenal glands, mammary glands, liver, spleen, lung, trachea or placenta are used.

21. A method of diagnosing or prognosing a disease, in particular a neurological disease, in a subject comprising:

determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of

(b) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f),

and comparing said level, or said activity, or both said level and said activity, of at least one of said substances (a) to (h) to a reference value representing a known disease or health status, thereby diagnosing or prognosing a disease, in particular a neurological disease, in said subject.

22. A method of monitoring the progression of a disease, in particular a neurological disease, in a subject, comprising:

determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f),

and comparing said level, or said activity, or both said level and said activity, of at least one of said substances (a) to (h) to a reference value representing a known disease or health status, thereby monitoring progression of a disease, in particular a neurological disease, in said subject.

23. A method of evaluating a treatment for a disease, in particular a neurological disease, in a subject, said method comprising:

determining a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f),

and comparing said level, or said activity, or both said level and said activity, of at least one of said substances (a) to (h) to a reference value representing a known disease or health status, thereby evaluating a treatment for a disease, in particular a neurological disease, in said subject.

24. The method according to one of claims 21 to 23, wherein the function of said protein molecule or a variant thereof is to protect cells from degeneration and/or cell death.

25. The method according to one of claims 21 to 24, wherein a decrease of a level or an activity of (i) a transcription product of a D N A molecule encoding a protein molecule, the amino acid sequence of which comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof or (ii) a protein molecule, the amino acid sequence of which comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof, in a sample from said subject relative to a reference value representing a known health status indicates the presence of a disease, in particular a neurological disease, in said subject.

26. The method according to one of claims 21 to 25, wherein said D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2

encodes a protein molecule, the function of which is to protect cells against degeneration and/or cell death.

27. The method according to one of claims 21 to 26, wherein said subject suffers from Alzheimer's disease or related neurofibrillary disorders, or neurodegenerative states characterized by cell degeneration or cell death, or Parkingson's disease, or Huntington disease, or Amyotrophic lateralsclerosis or Pick's disease.

28. An agent which affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f).

29. An agent of claim 28, wherein the function of said protein molecule or a variant thereof is to protect cells from degeneration and/or cell death.

30. An agent of claim 28 or 29 wherein said D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 encodes a protein molecule, the function of which is to protect cells against degeneration and/or cell death.

31. A medicament comprising an agent according to one of claims 28 to 30.

32. Use of an agent for preparation of a medicament for treating or preventing a neurological disease, in particular Alzheimer's disease, which agent affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f).

33. Use of an agent according to claim 32, wherein the function of said protein molecule or a variant thereof is to protect cells from degeneration and/or cell death.

34. Use of an agent according to claim 32 or 33, wherein said D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 encodes a protein molecule, the function of which is to protect cells against degeneration and/or cell death.

35. A method of identifying an agent that affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f),

comprising the steps of:

(i) providing a sample containing at least one substance which is selected from the group consisting of (a) to (f),

(ii) contacting said sample with at least one agent,

(iii) comparing an activity, or level, or both said activity and level, of at least one of said substances before and after contacting.

36. A method of claim 35 wherein the function of said protein molecule or a variant thereof is to protect cells from degeneration and/or cell death.

37. A method of claim 35 or 36 wherein said D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 encodes a protein molecule, the function of which is to protect cells against degeneration and/or cell death.

38. A kit for diagnosis, or prognosis of a disease, said kit comprising:

(1) at least one reagent which is selected from the group consisting of reagents that selectively detect

(a) a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(b) a transcription product of a D N A molecule encoding a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(c) a protein molecule, wherein the amino acid sequence of the protein molecule comprises the sequence shown in SEQ ID NO.1 or a functional variant thereof,

(d) a D N A molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(e) a transcription product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(f) a translation product of a D N A molecule, wherein said D N A molecule is capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 under stringent conditions,

(g) a molecule affecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (f),

(h) a molecule which is affected in its level, or its activity, or both its level and activity, by at least one substance which is selected from the group consisting of (a) to (f),

(2) instructions for diagnosing, or prognosing said disease by

(i) detecting a level, or an activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (h) in a sample from said subject;

and

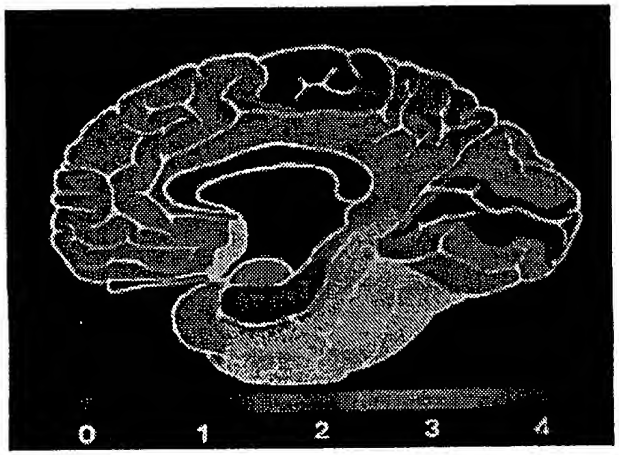
(ii) diagnosing, or prognosing said disease, wherein

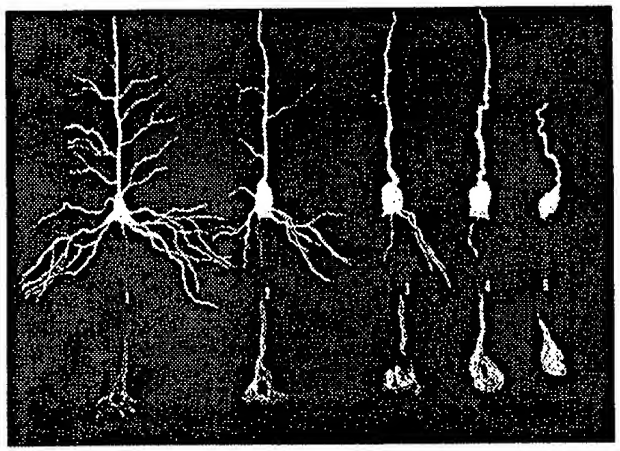
a varied level, or activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (h) compared to a reference value representing a known health status;

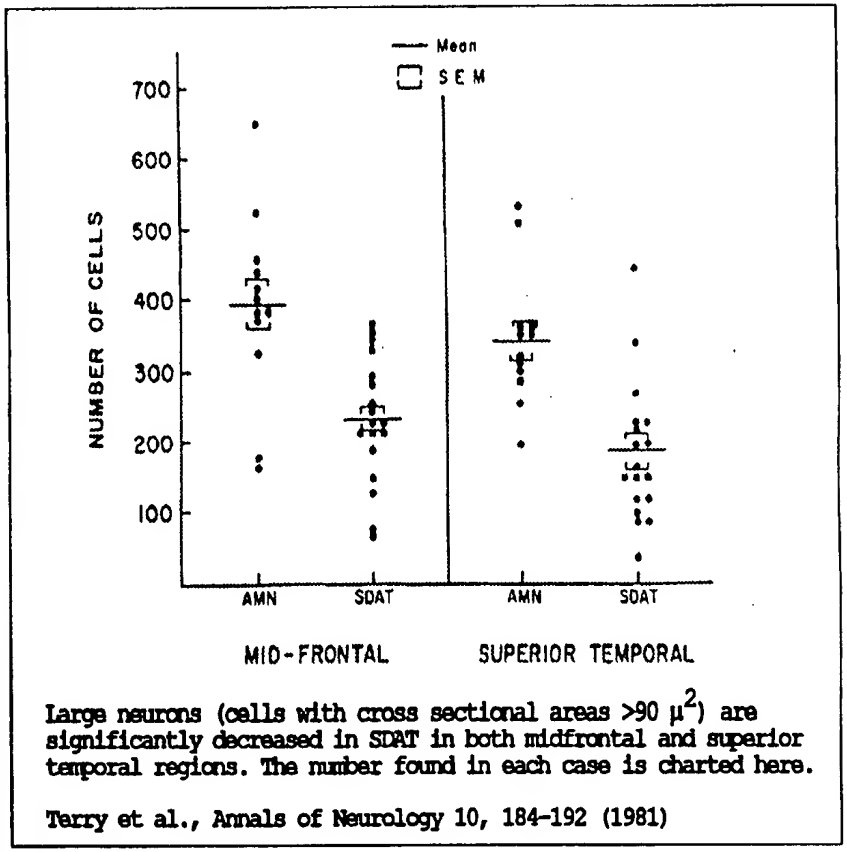
or a level, or activity, or both said level and said activity, of at least one substance which is selected from the group consisting of (a) to (h) similar or equal to a

reference value representing a known disease status indicates diagnosis, or prognosis of said disease.

Selective vulnerability of brain regions in Alzheimer´s disease







Large neurons (cells with cross sectional areas >90 μ^2) are significantly decreased in SDAT in both midfrontal and superior temporal regions. The number found in each case is charted here.

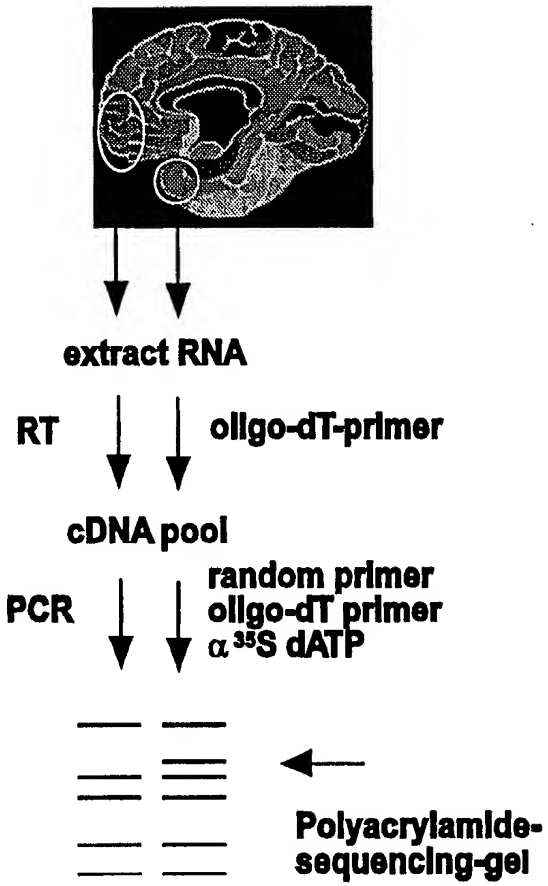
Terry et al., Annals of Neurology 10, 184-192 (1981)

Figure 1

WO 00/29569

PCT/EP99/08744

Identification of genes differentially expressed in AD brain regions



- cut out differentially expressed bands
- reamplify eluted cDNAs
- cloning and sequencing
- expression analysis
- functional analysis

Figure 2

Identification of genes differentially expressed in AD brain regions

---

Material: AD brain tissue

post mortem time intervall <6h

2 different regions histologically characterized

- inferior temporal lobe
- frontal cortex

Method: mRNA differential display screen

Figure 3

Expression of Seladin-1 in AD brain

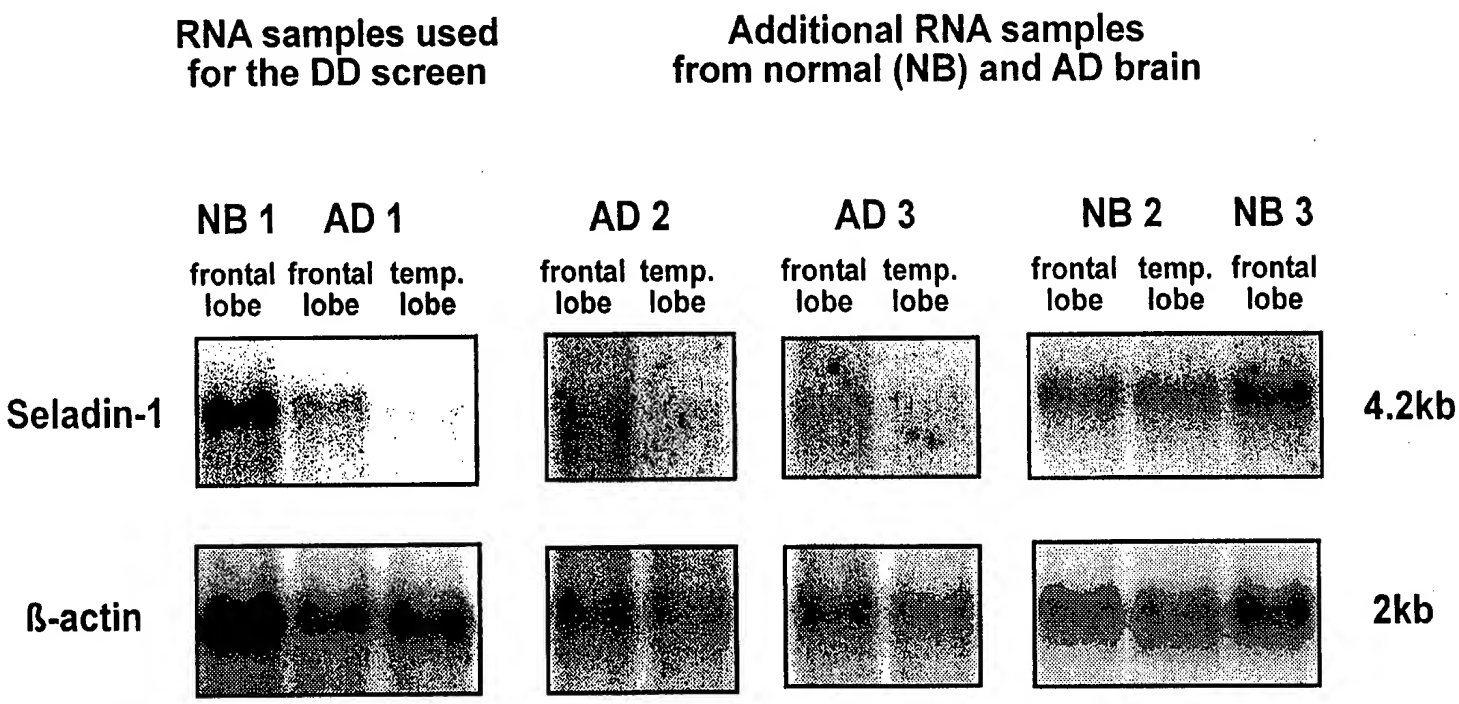


Figure 4

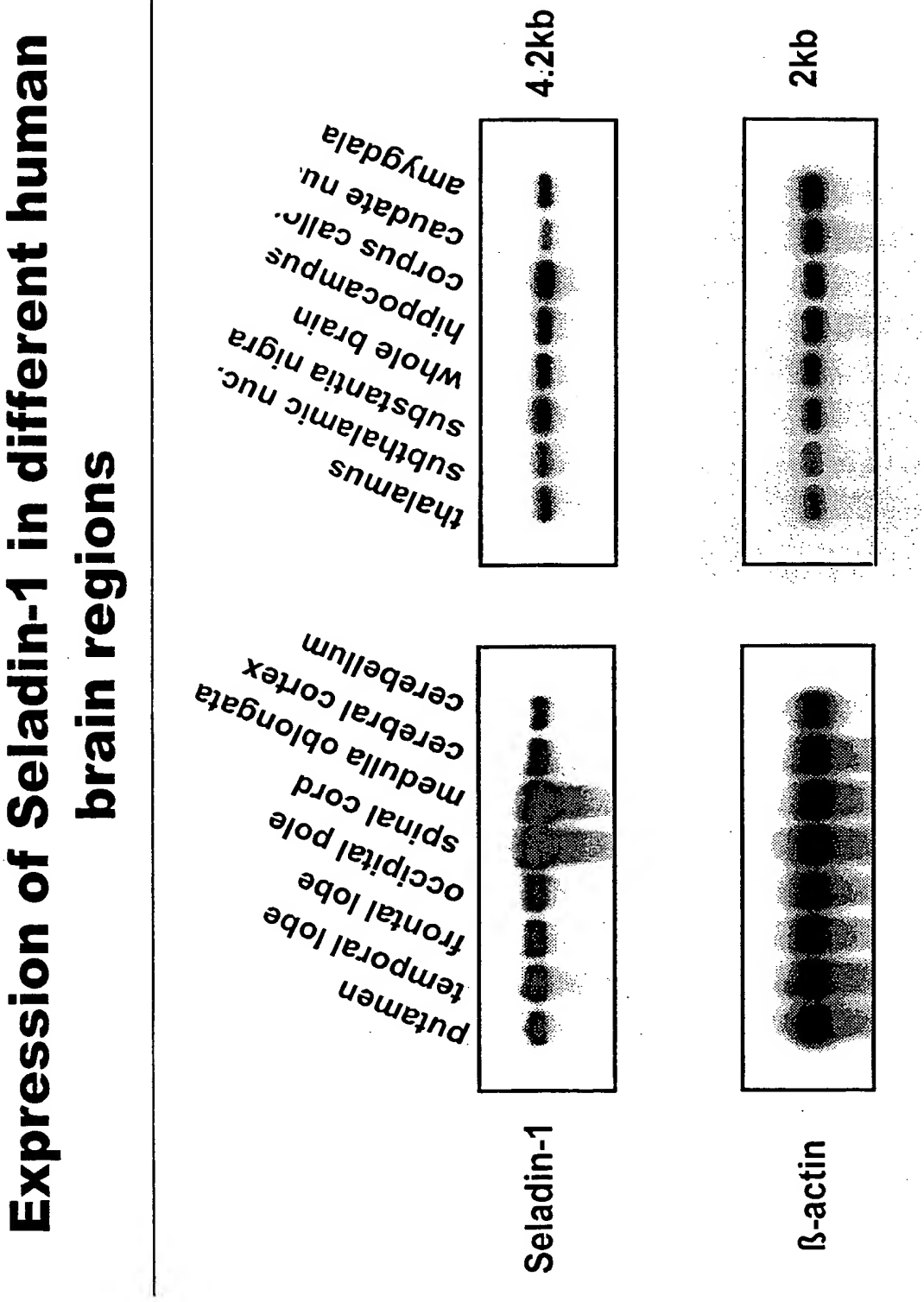
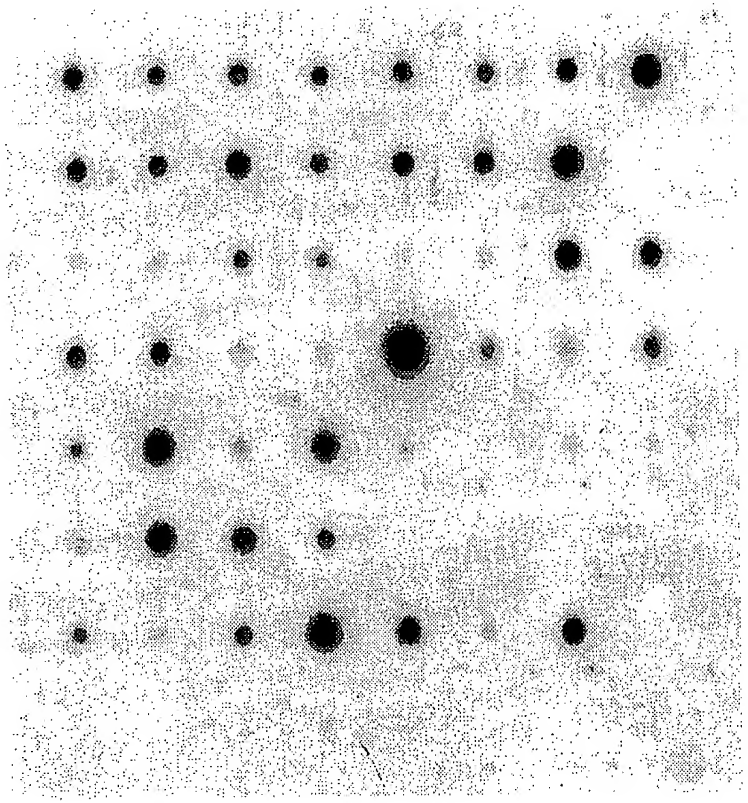

Expression of Seladin-1 in different human brain regions
4.2kb
2kb
thalamus
subthalamic nuc.
substantia nigra
whole brain
hippocampus
corpus callo.
caudate nu.
amygdala
putamen
temporal lobe
frontal lobe
occipital pole
spinal cord
medulla oblongata
cerebral cortex
cerebellum
Seladin-1
ß-actin

Figure 5

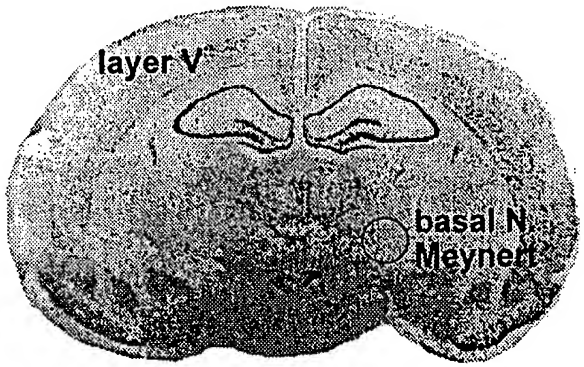
Expression of Seladin-1 in human tissues

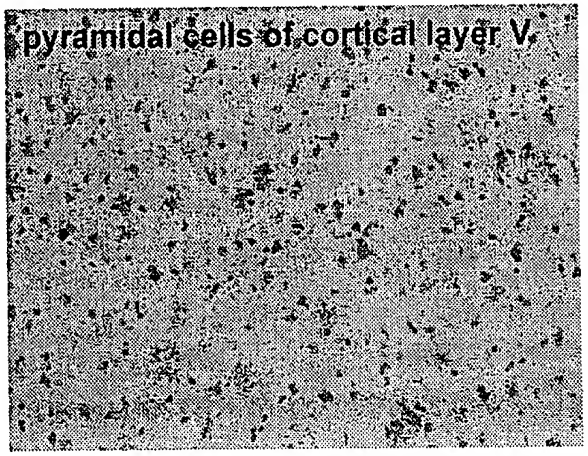


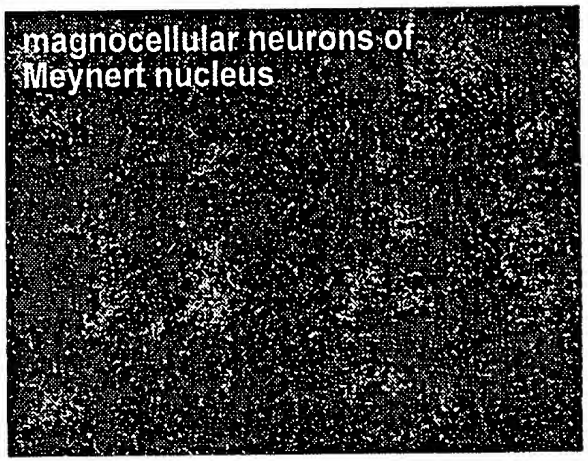
whole brain	amygdala	caudate nucleus	cerebellum	cerebral cortex	frontal lobe	hippocampus	medulla oblongata
occipital lobe	putamen	substantia nigra	temporal lobe	thalamus	subthalamic nucleus	spinal cord	
heart	aorta	skeletal muscle	colon	bladder	uterus	prostate	stomach
testis	ovary	pancreas	pituitary gland	adrenal gland	thyroid gland	salivary gland	mammary gland
kidney	liver	small intestine	spleen	thymus	peripheral leukocyte	lymph node	bone marrow
appendix	lung	trachea	placenta				
fetal brain	fetal heart	fetal kidney	fetal liver	fetal spleen	fetal thymus	fetal lung	
yeast total RNA 100ng	yeast tRNA 100ng	E. coli rRNA 100ng	E. coli DNA 100ng	Poly r(A) 100ng	human Cot1DNA 100ng	human DNA 100ng	human DNA 500ng

Figure 6

Expression of Seladin-1 in rat brain







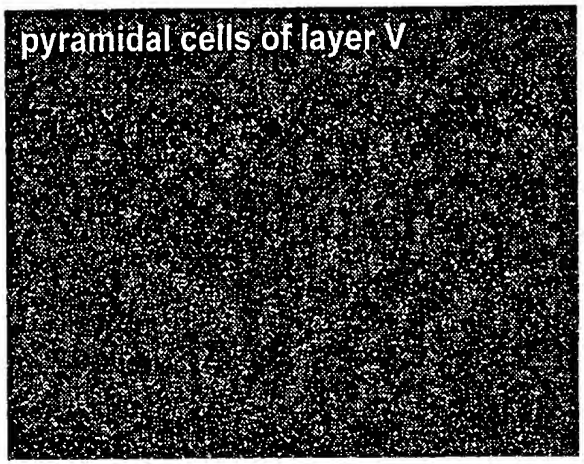


Figure 7

Figure 8

Expression of Seladin-1 in rat brain

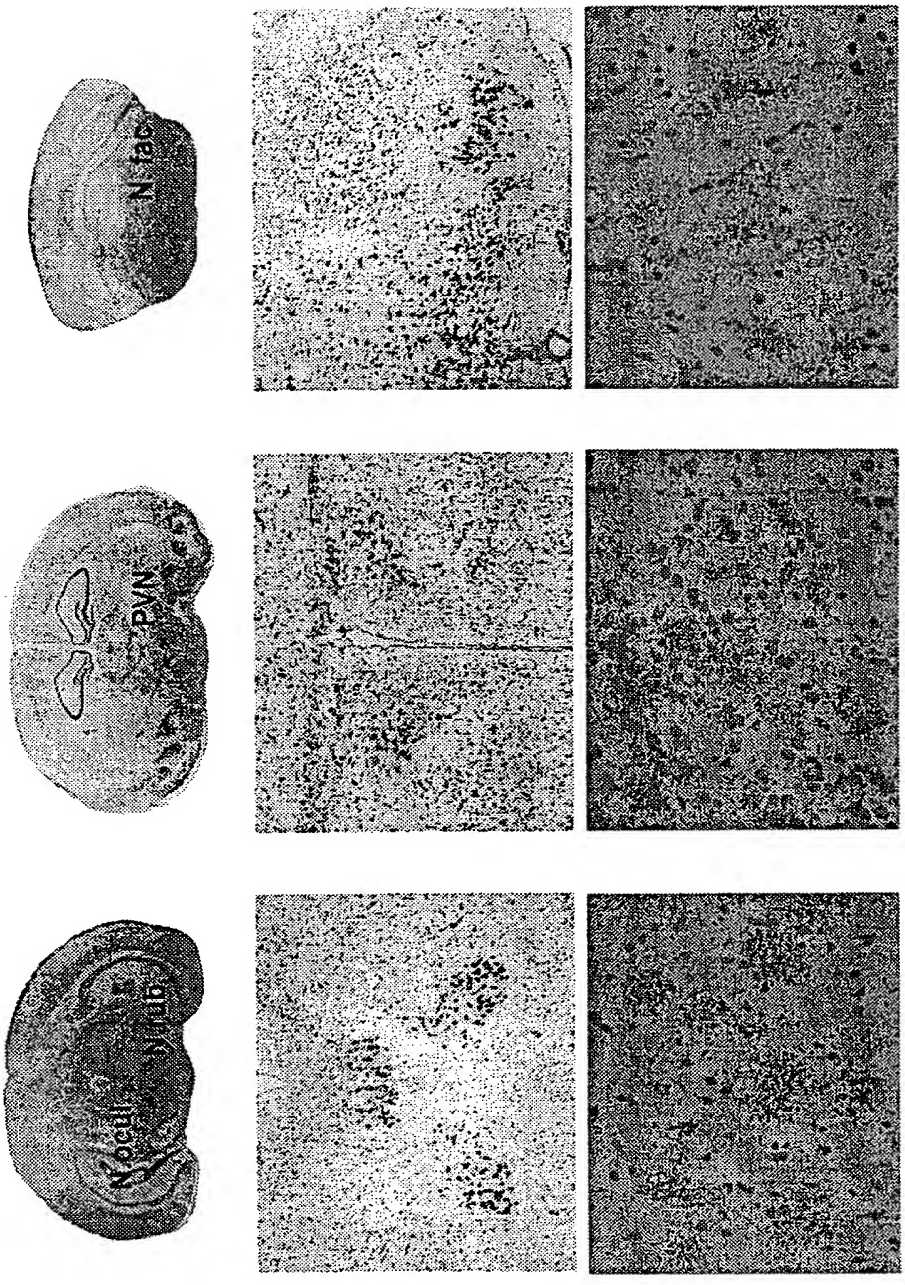
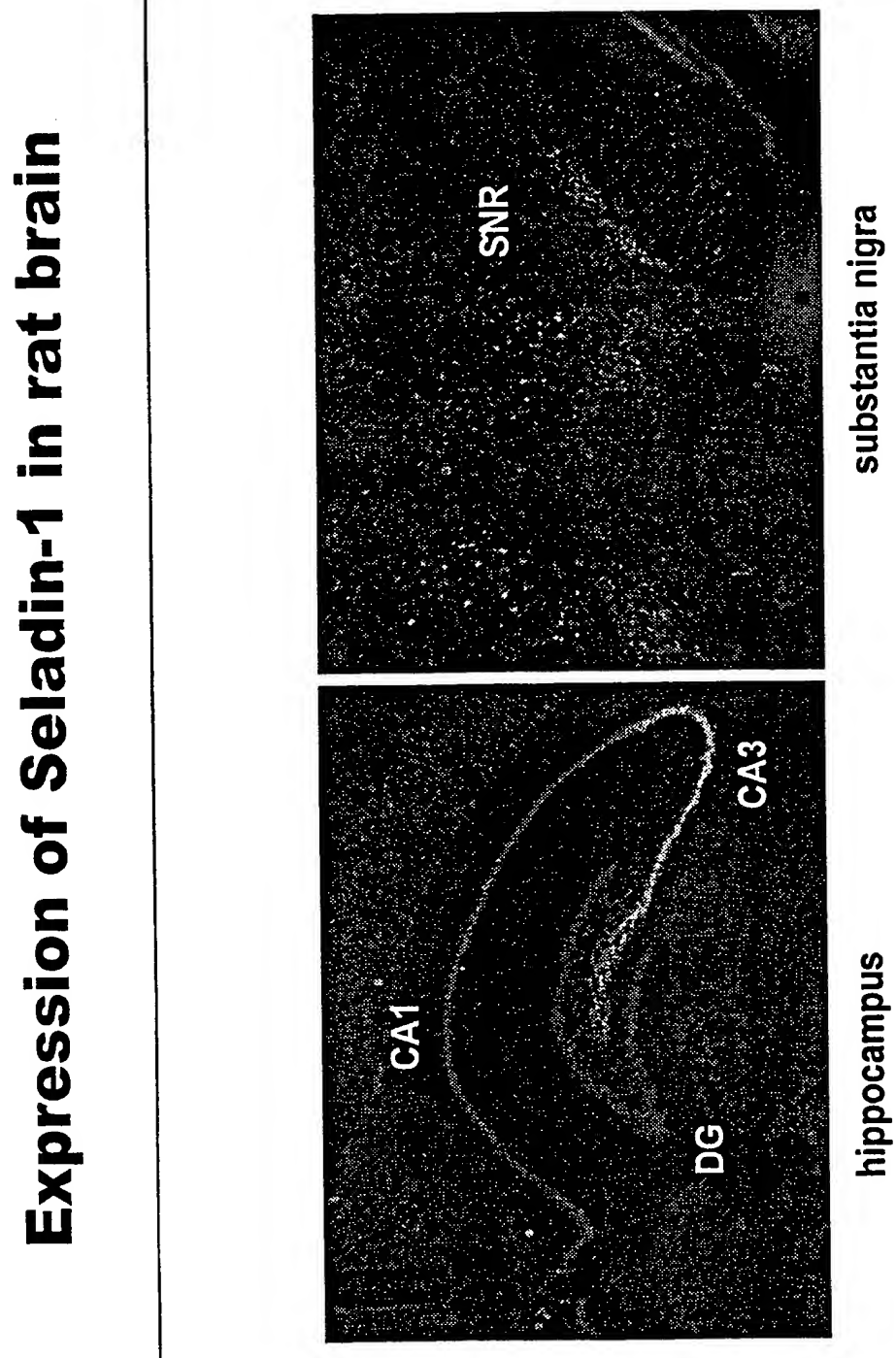


Figure 9


Expression of Seladin-1 in rat brain
SNR
substantia nigra
CA1
CA3
DG
hippocampus

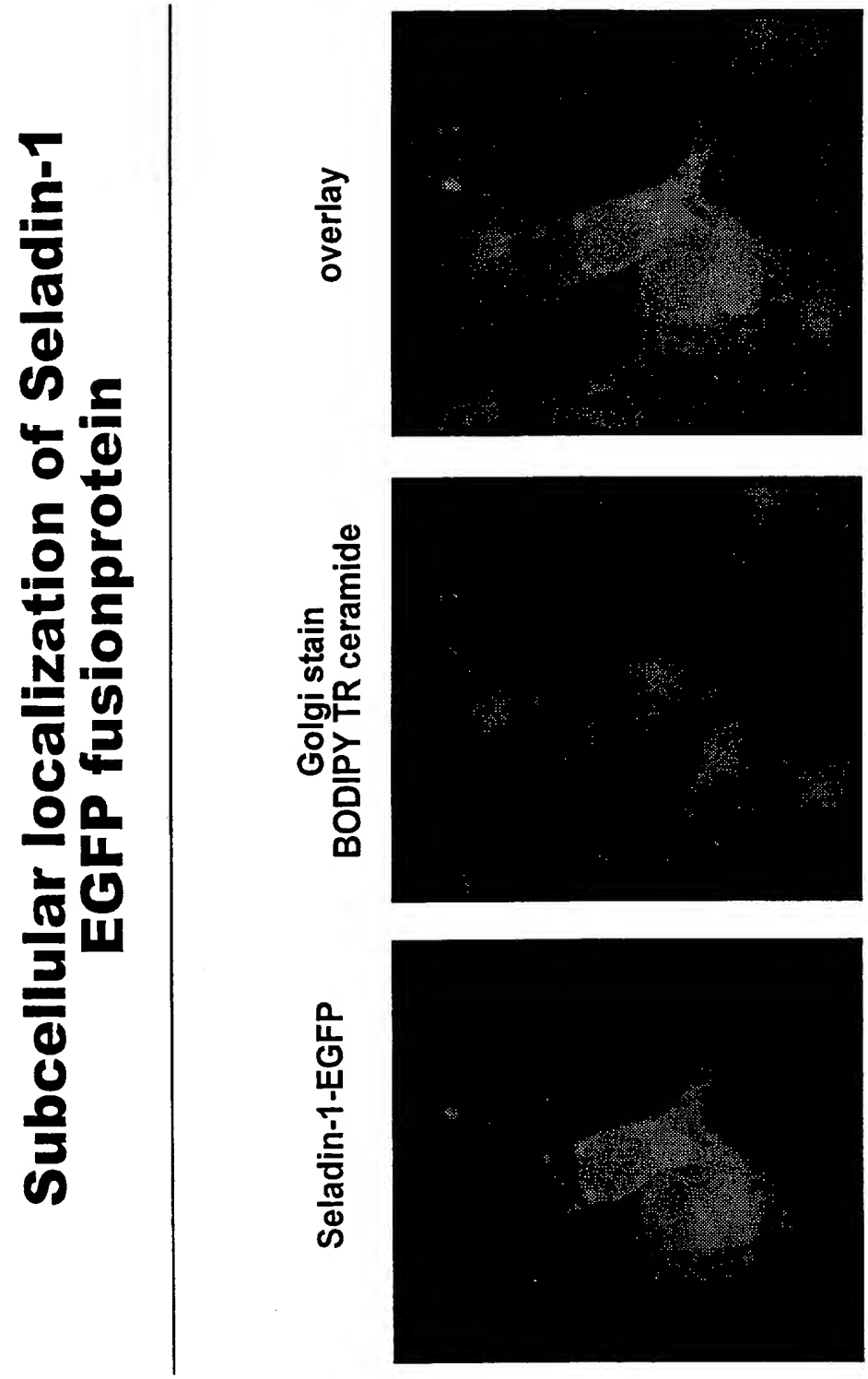

Subcellular localization of Seladin-1
EGFP fusionprotein
Seladin-1-EGFP
Golgi stain
BODIPY TR ceramide
overlay

Figure 10

Subcellular localization of Seladin-1 EGFP fusionprotein

Seladin-1-EGFP

Mitochondrial stain
MitoTracker Red CM-H2XRos

overlay

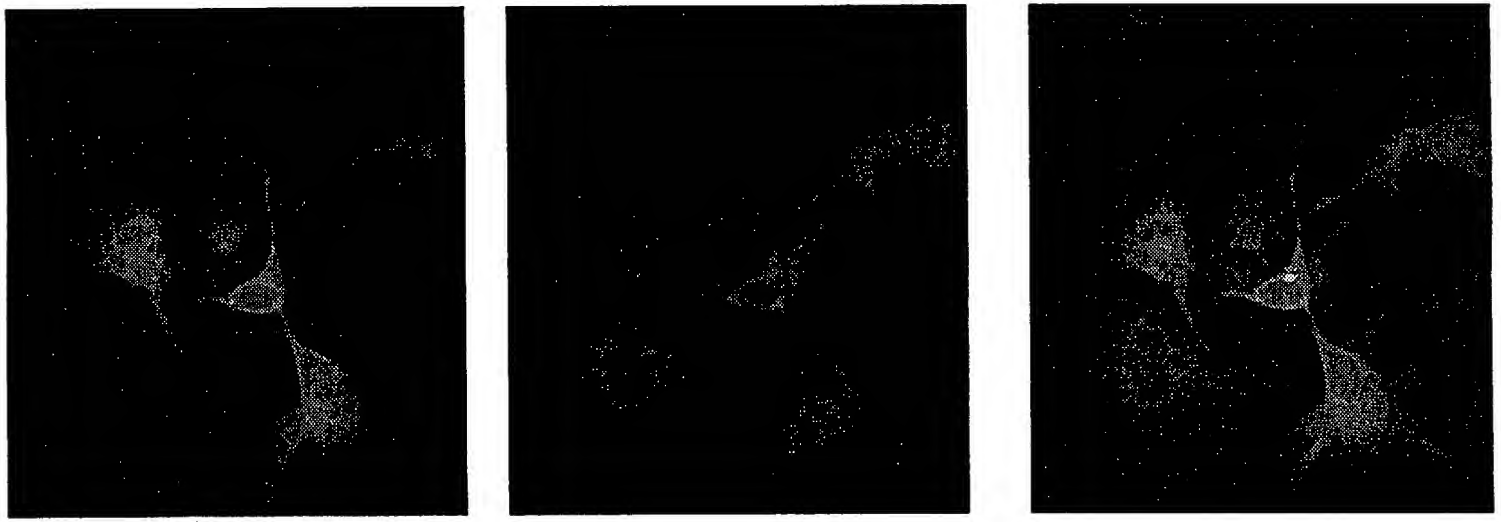
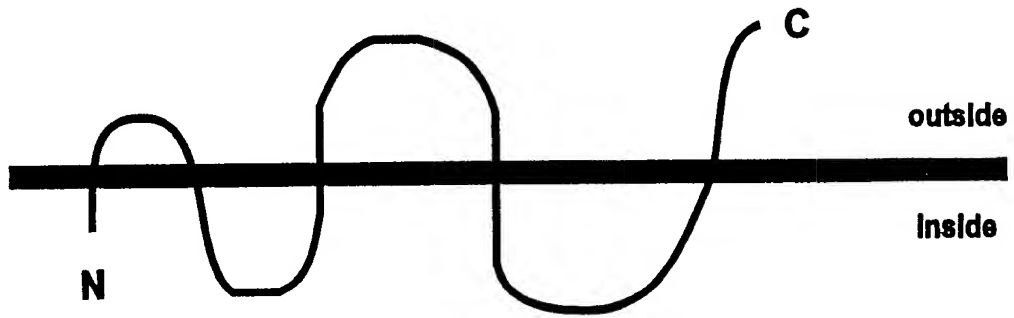


Figure 11

Multiple sequence alignments and secondary structure prediction of Seladin-1

- putative mitochondrial targeting sequence
- homology region to many oxido-reductases
  e.g. L-gulono-gamma-lactone-oxidase
  6-hydroxy-d-nicotine oxidase
  D-lactate dehydrogenase
- contains oxygen oxidoreductases covalent FAD binding site



- 5 possible transmembrane regions

Figure 12

FIGURE 13: SEQ ID NO.1

Seladin-1 amino acid sequence

Seladin-1.orf Length: 516 May 29, 1998 14:51 Type: P
Check: 1354 ..

1 MEPAVSLAVC ALLFLLWVRL KGLEFVLIHQ RWVFVCLFLL PLSLIFDIYY

51 YVRAWVVFKL SSAPRLHEQR VRDIQKQVRE WKEQGSKTFM CTGRPGWLTV

101 SLRVGKYKKT HKNIMINLMD ILEVDTKKQI VRVEPLVTMG QVTALLTSIG

151 WTLPVLPELD DLTVGGLIMG TGIESSSHKY GLFQHICTAY ELVLADGSFV

201 RCTPSENSDL FYAVPWSCGT LGFLVAAEIR IIPAKKYVKL RFEPVRGLEA

251 ICAKFTHESQ RQENHFVEGL LYSLDEAVIM TGVMTDEAEP SKLNSIGNYY

301 KPWFFKHVEN YLKTNREGLE YIPLRHYYHR HTRSIFWELQ DIIPFGNNPI

351 FRYLFGWMVP PKISLLKLTQ GETLRKLYEQ HHVVQDMLVP MKCLQQALHT

401 FQNDIHVYPI WLCPFILPSQ PGLVHPKGNE AELYIDIGAY GEPRVKHFEA

451 RSCMRQLEKF VRSVHGFQML YADCYMNREE FWEMFDGSLY HKLREKLGCQ

501 DAFPEVYDKI CKAARH

FIGURE 14: SEQ ID NO.2

Seladin-1 cDNA sequence

Seladin-1 Length: 4248 April 28, 1998 14:10 Type: N Check: 8184 ..

```
   1 cccgggctgt gggctacagg cgcagagcgg gccaggcgcg gagctggcgg
  51 cagtgacagg aggcgcgaac ccgcagcgct taccgcgcgg cgccgcacca
 101 tggagcccgc cgtgtcgctg gccgtgtgcg cgctgctctt cctgctgtgg
 151 gtgcgcctga aggggctgga gttcgtgctc atccaccagc gctgggtgtt
 201 cgtgtgcctc ttcctcctgc cgctctcgct tatcttcgat atctactact
 251 acgtgcgcgc ctgggtggtg ttcaagctca gcagcgctcc gcgcctgcac
 301 gagcagcgcg tgcgggacat ccagaagcag gtgcgggaat ggaaggagca
 351 gggtagcaag accttcatgt gcacggggcg ccctggctgg ctcactgtct
 401 cactacgtgt cgggaagtac aagaagacac acaaaaacat catgatcaac
 451 ctgatggaca ttctggaagt ggacaccaag aaacagattg tccgtgtgga
 501 gcccttggtg accatgggcc aggtgactgc cctgctgacc tccattggct
 551 ggactctccc cgtgttgcct gagcttgatg acctcacagt gggggggcttg
 601 atcatgggca caggcatcga gtcatcatcc cacaagtacg gcctgttcca
 651 acacatctgc actgcttacg agctggtcct ggctgatggc agctttgtgc
 701 gatgcactcc gtccgaaaac tcagacctgt tctatgccgt accctggtcc
 751 tgtgggacgc tgggtttcct ggtggccgct gagatccgca tcatccctgc
 801 caagaagtac gtcaagctgc gtttcgagcc agtgcggggc ctggaggcta
 851 tctgtgccaa gttcacccac gagtcccagc ggcaggagaa ccacttcgtg
 901 gaagggctgc tctactccct ggatgaggct gtcattatga caggggtcat
 951 gacagatgag gcagagccca gcaagctgaa tagcattggc aattactaca
1001 agccgtggtt ctttaagcat gtggagaact atctgaagac aaaccgagag
1051 ggcctggagt acattccctt gagacactac taccaccgcc acacgcgcag
1101 catcttctgg gagctccagg acatcatccc ctttggcaac aaccccatct
1151 tccgctacct ctttggctgg atggtgcctc ccaagatctc cctcctgaag
1201 ctgacccagg gtgagaccct gcgcaagctg tacgagcagc accacgtggt
1251 gcaggacatg ctggtgccca tgaagtgcct gcagcaggcc ctgcacacct
1301 tccaaaacga catccacgtc taccccatct ggctgtgtcc gttcatcctg
1351 cccagccagc caggcctagt gcaccccaaa ggaaatgagg cagagctcta
```

1401 catcgacatt ggagcatatg gggagccgcg tgtgaaacac tttgaagcca

1451 ggtcctgcat gaggcagctg gagaagtttg tccgcagcgt gcatggcttc

1501 cagatgctgt atgccgactg ctacatgaac cgggaggagt tctgggagat

1551 gtttgatggc tccttgtacc acaagctgcg agagaagctg ggttgccagg

1601 acgccttccc cgaggtgtac gacaagatct gcaaggccgc caggcactga

1651 gctggagccc gcctggagag acagacacgt gtgagtggtc aggcatcttc

1701 ccttcactca agcttggctg ctttcctaga tccacacttt caaagagaaa

1751 cccctccaga actcccaccc tgacagccca acaccacctt cctcctggct

1801 tccagggggc agcccagtgg aatggaaaga atgtgggatt tggagtcaga

1851 caagcctgag tccagttccc cgtttagaac tcattagctg tgtgactctg

1901 ggtgagtccc ttaacccctc tgagcccggg tctcttcatt agttgaaagg

1951 gatagtaata cctacttgca ggttgttgtc atctgagttg agcactggtc

2001 acattgaagg tgctgggtaa gtggtagctc ttgttgcttc ccgttcagcg

2051 tcacatctgc agtggagcct gaaaaggctc cacattaggt cacctgtgca

2101 cagccatggc tggaatgatg aaggggatac gctggagttg ccctgccatc

2151 gcctccatca gccagacgag gtcctcacag gagaaggaca gctcttcccc

2201 accctgggat ctcaggaggg cagccacgga gtggggaggc cccagatgcg

2251 ctgtgccaaa gccaggtccg aggccaaagt tctccctgcc atccttggtg

2301 ccgtcctgcc ccttcctcct tcatgcctgg gcctgcaggc ccaccccagc

2351 caccactgag tccactcgga gtgccctgtg ttcctggaga aggcattcca

2401 gggttgaatc ttgtcccagc ctcagcctgg gacacctagg tggagagagt

2451 ggtctccgct ctgaattgga tccaggggac ctgggctcat tcttcttggc

2501 tcaccaaccc tgcaggcctc atctttccca aaacccactt tgtcttggtg

2551 ggagtgggtc cgcgctgctc tgcagcaggg gctggggagt ggacagcatc

2601 acgtgggaaa gtggagtcca ccctcatgtt tctgtaggat tctcaccgtg

2651 gggctggaag aaaagagcat cgacttgatt tctccaacca ctcatccctc

2701 tttttctttc ttccaccact ccccacccca gctgtagtta atttcagtgc

2751 cttacaaatc ctaagctcag agaaagttcc atttccgttc cagagggaag

2801 ggaacctccc taggtccttc cctggcttgt tataacgcaa agcttggttg

2851 tttatgcaac tctatcttaa gaactgccca gcctcagctg aaaacccgaa

2901 tctgagaagg aattgcgtca tgtaagggaa gctggaatta agggagctga

2951 gccagtcatg gttgtggcgt gtgagtcagg agacctaggt ttcagcccct

3001 ctctactgtc agcgagctgt gcaacgtggg caagtcattg tcctctgagc

3051 tgcagtttcc tcatctgtca catcgctaca gacaagacct ccctggaacc

3101 cttctgattg tcttagacac tgtggttgca aaacccacgg aaagcctcat

3151 ttgtgtggaa agtcagagga aaaatgatcc agtggacact tggggattat

3201 ctgtcattca agatccttcc ttcaacccca aggccagctc ccatctcatt

3251 tccagaaagg ctcatacctg gcttgcaggg aagcatctgt cttgtcattc

3301 caggtgccag aatcctctca gagtcattga agggtgttca cccatcccac

3351 ccaaggcttg gcacactgcc agtgtcttag cagggtcttg tgagggctgg

3401 gggcatccag gcactcagaa ggcaaaggaa ccaccctacc catttggcct

3451 ctggaggggg cagaagaaag aaagaaacct catcctatat tttacaaagc

3501 atgtgaattc tggcattagc tctcatagga gacccatgtg cttccttgct

3551 cagtgcaaaa ctgatgattc tacttgctgt agatgaatgg ttaacacgag

3601 ctagttaaac agtgccattg ttttgccagt gaagcctcca accctaagcc

3651 actgggacgg tggccagaga tgccagcagc ctctgtcgcc cttagtcata

3701 taaccaaaat ccagacctta tccacaaccc ggggcttgga aaggaaggta

3751 ttttggaatc acaccctccg gttatgttgc tccagtaaaa tcttgcctgg

3801 aaagaggcag tcttcttagc atggtgagct gagttcatgg ctttttttg

3851 tagccagtcc tgtccctggc catccatgtg atggttttgg atggagttaa

3901 acttgatgcc agtgggcagt gcatgtggaa agtatcagag taagcctctc

3951 ccctccagag ccctgagttt cttggctgca tgaaggtttt ctttagaatc

4001 agaattgtag ccagtttctt tggccagaag gatgaatact tggatattac

4051 tgaaagggag gggtggagat gggtgtggca gtgtatggtg tgtgattttt

4101 attttcttct ttggtcatgg gggccaagga gaaaggcatg aatcttccct

4151 gtcaggctct tacagccaca ggcactgtgt ctactgtctg gaagacatgt

4201 ccccgtggct gtggggccgc tgcttctgtt taaataaaag tggcctgg

FIGURE 15 CDNA sequence comparison KIAA0018/Seladin-1

```
    1 ggcgcgaacccgcagcgcttaccgcgcggcgccgcaccatggagcccgcc 50
      ||||||||||||||||||||||||||||||||||||||||||||||||||
   62 ggcgcgaacccgcagcgcttaccgcgcggcgccgcaccatggagcccgcc 111

   51 gtgtcgctggccgtgtgcgcgctgctcttcctgctgtgggtgcgcctgaa 100
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  112 gtgtcgctggccgtgtgcgcgctgctcttcctgctgtgggtgcgcctgaa 161

  101 ggggctggagttcgtgctcatccaccagcgctgggtgttcgtgtgcctct 150
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  162 ggggctggagttcgtgctcatccaccagcgctgggtgttcgtgtgcctct 211

  151 tcctcctgccgctctcgcttatcttcgatatctactactacgtgcgcgcc 200
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  212 tcctcctgccgctctcgcttatcttcgatatctactactacgtgcgcgcc 261

  201 tgggtggtgttcaagctcagcagcgctccgcgcctgcacgagcagcgcgt 250
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  262 tgggtggtgttcaagctcagcagcgctccgcgcctgcacgagcagcgcgt 311

  251 gcgggacatccagaagcaggtgcgggaatggaaggagcagggtagcaaga 300
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  312 gcgggacatccagaagcaggtgcgggaatggaaggagcagggtagcaaga 361

  301 ccttcatgtgcacggggcgccctggctggctcactgtctcactacgtgtc 350
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  362 ccttcatgtgcacggggcgccctggctggctcactgtctcactacgtgtc 411

  351 gggaagtacaagaagacacacaaaaacatcatgatcaacctgatggacat 400
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  412 gggaagtacaagaagacacacaaaaacatcatgatcaacctgatggacat 461

  401 tctggaagtggacaccaagaaacagattgtccgtgtggagcccttggtga 450
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  462 tctggaagtggacaccaagaaacagattgtccgtgtggagcccttggtga 511

  451 ccatgggccaggtgactgccctgctgacctccattggctggactctcccc 500
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  512 ccatgggccaggtgactgccctgctgacctccattggctggactctcccc 561

  501 gtgttgcctgagcttgatgacctcacagtgggggggcttgatcatgggcac 550
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  562 gtgttgcctgagcttgatgacctcacagtgggggggcttgatcatgggcac 611

  551 aggcatcgagtcatcatcccacaagtacggcctgttccaacacatctgca 600
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  612 aggcatcgagtcatcatcccacaagtacggcctgttccaacacatctgca 661

  601 ctgcttacgagctggtcctggctgatggcagctttgtgcgatgcactccg 650
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  662 ctgcttacgagctggtcctggctgatggcagctttgtgcgatgcactccg 711

  651 tccgaaaactcagacctgttctatgccgtaccctggtcctgtgggacgct 700
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  712 tccgaaaactcagacctgttctatgccgtaccctggtcctgtgggacgct 761

  701 gggtttcctggtggccgctgagatccgcatcatccctgccaagaagtacg 750
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  762 gggtttcctggtggccgctgagatccgcatcatccctgccaagaagtacg 811
```

```
 751 tcaagctgcgtttcgagccagtgcggggcctggaggctatctgtgccaag 800
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
 812 tcaagctgcgtttcgagccagtgcggggcctggaggctatctgtgccaag 861

 801 ttcacccacgagtcccagcggcaggagaaccacttcgtggaagggctgct 850
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
 862 ttcacccacgagtcccagcggcaggagaaccacttcgtggaagggctgct 911

 851 ctactccctggatgaggctgtcattatgacaggggtcatgacagatgagg 900
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
 912 ctactccctggatgaggctgtcattatgacaggggtcatgacagatgagg 961

 901 cagagcccagcaagctgaatagcattggcaattactacaagccgtggttc 950
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
 962 cagagcccagcaagctgaatagcattggcaattactacaagccgtggttc 1011

 951 tttaagcatgtggagaactatctgaagacaaaccgagagggcctggagta 1000
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1012 tttaagcatgtggagaactatctgaagacaaaccgagagggcctggagta 1061

1001 cattcccttgagacactactaccaccgccacacgcgcagcatcttctggg 1050
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1062 cattcccttgagacactactaccaccgccacacgcgcagcatcttctggg 1111

1051 agctccaggacatcatccccctttggcaacaaccccatcttccgctacctc 1100
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1112 agctccaggacatcatccccctttggcaacaaccccatcttccgctacctc 1161

1101 tttggctggatggtgcctcccaagatctccctcctgaagctgacccaggg 1150
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1162 tttggctggatggtgcctcccaagatctccctcctgaagctgacccaggg 1211

1151 tgagaccctgcgcaag.tgtacgagcagcaccacgtggtgcaggacatgc 1199
     |||||||||||||||| ||||||||||||||||||||||||||||||||||
1212 tgagaccctgcgcaagctgtacgagcagcaccacgtggtgcaggacatgc 1261

1200 tggtgcccatgaagtgcctgcagcaggccctgcacaccttccaaaacgac 1249
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1262 tggtgcccatgaagtgcctgcagcaggccctgcacaccttccaaaacgac 1311

1250 atccacgtctaccccatctggctgtgtccgttcatcctgcccagccagcc 1299
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1312 atccacgtctaccccatctggctgtgtccgttcatcctgcccagccagcc 1361

1300 aggcctagtgcaccccaaaggaaatgaggcagagctctacatcgacattg 1349
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1362 aggcctagtgcaccccaaaggaaatgaggcagagctctacatcgacattg 1411

1350 gagcatatggggagccgcgtgtgaaacactttgaagccaggtcctgcatg 1399
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1412 gagcatatggggagccgcgtgtgaaacactttgaagccaggtcctgcatg 1461

1400 aggcagctggagaagtttgtccgcagcgtgcatggcttccagatgctgta 1449
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1462 aggcagctggagaagtttgtccgcagcgtgcatggcttccagatgctgta 1511

1450 tgccgactgctacatgaaccgggaggagttctgggagatgtttgatggct 1499
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1512 tgccgactgctacatgaaccgggaggagttctgggagatgtttgatggct 1561

1500 ccttgtaccacaagctgcgagagaagctgggttgccaggacgccttcccc 1549
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
1562 ccttgtaccacaagctgcgagagaagctgggttgccaggacgccttcccc 1611
```

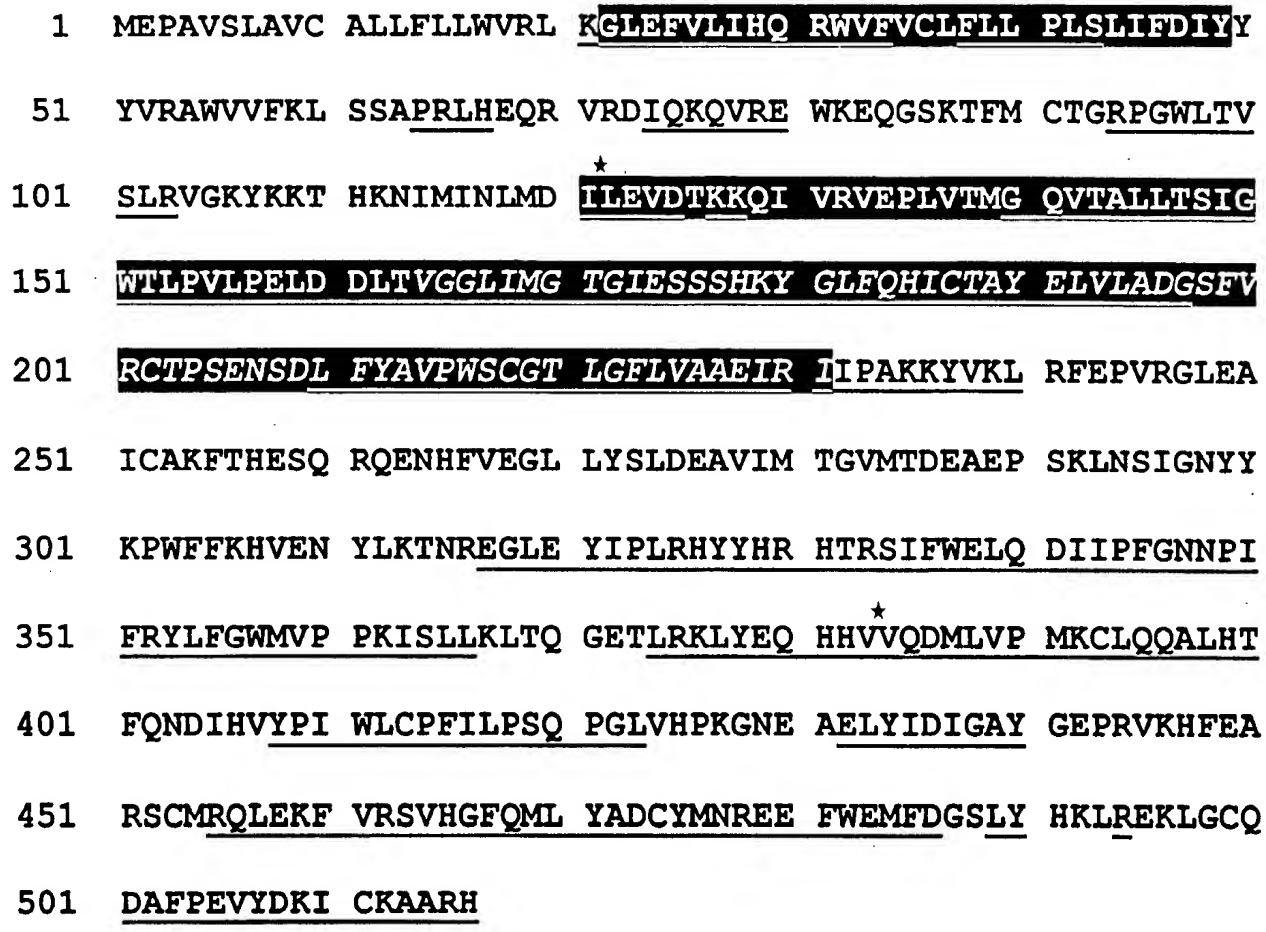
```
1550 gaggtgtacgacaagatctgcaaggccgccaggcactgagctggagcccg 1599
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1612 gaggtgtacgacaagatctgcaaggccgccaggcactgagctggagcccg 1661

1600 cctggagagacagacacgtgtgagtggtcaggcatcttcccttcactcaa 1649
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1662 cctggagagacagacacgtgtgagtggtcaggcatcttcccttcactcaa 1711

1650 gcttggctgctttcctagatccacactttcaaagagaaaccccctccagaa 1699
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1712 gcttggctgctttcctagatccacactttcaaagagaaaccccctccagaa 1761

1700 ctcccaccctgacagcccaacaccaccttcctcctggcttccaggggcca 1749
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1762 ctcccaccctgacagcccaacaccaccttcctcctggcttccaggggcca 1811

1750 gcccagtggaatggaaagaatgtgggatttggagtcagacaagcctgagt 1799
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1812 gcccagtggaatggaaagaatgtgggatttggagtcagacaagcctgagt 1861

1800 ccagttccccgtttagaactcattagctgtgtgactctgggtgagtccct 1849
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1862 ccagttccccgtttagaactcattagctgtgtgactctgggtgagtccct 1911

1850 taacccctctgagcccgggtctcttcattagttgaaagggatagtaatac 1899
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1912 taacccctctgagcccgggtctcttcattagttgaaagggatagtaatac 1961

1900 ctacttgcaggttgttgtcatctgagttgagcactggtcacattgaaggt 1949
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1962 ctacttgcaggttgttgtcatctgagttgagcactggtcacattgaaggt 2011

1950 gctgggtaagtggtagctcttgttgcttcccgttcagcgtcacatctgca 1999
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2012 gctgggtaagtggtagctcttgttgcttcccgttcagcgtcacatctgca 2061

2000 gtggagcctgaaaaggctccacattaggtcacctgtgcacagccatggct 2049
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2062 gtggagcctgaaaaggctccacattaggtcacctgtgcacagccatggct 2111

2050 ggaatgatgaaggggatacgctggagttgccctgccatcgcctccatcag 2099
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2112 ggaatgatgaaggggatacgctggagttgccctgccatcgcctccatcag 2161

2100 ccagacgaggtcctcacaggagaaggacagctcttccccaccctgggatc 2149
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2162 ccagacgaggtcctcacaggagaaggacagctcttccccaccctgggatc 2211

2150 tcaggagggcagccacggagtggggaggccccagatgcgctgtgccaaag 2199
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2212 tcaggagggcagccacggagtggggaggccccagatgcgctgtgccaaag 2261

2200 ccaggtccgaggccaaagttctccctgccatccttggtgccgtcctgccc 2249
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2262 ccaggtccgaggccaaagttctccctgccatccttggtgccgtcctgccc 2311

2250 cttcctccttcatgcctgggcctgcaggcccaccccagccaccactgagt 2299
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2312 cttcctccttcatgcctgggcctgcaggcccaccccagccaccactgagt 2361

2300 ccactcggagtgccctgtgttcctggagaaggcattccagggttgaatct 2349
     ||||||||||||||||||||||||||||||||||||||||||||||||||
2362 ccactcggagtgccctgtgttcctggagaaggcattccagggttgaatct 2411
```

```
2350 tgtcccagcctcagcctgggacacctaggtggagagagtggtctccgctc 2399
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2412 tgtcccagcctcagcctgggacacctaggtggagagagtggtctccgctc 2461

2400 tgaattggatccaggggacctgggctcattcttcttggctcaccaaccct 2449
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2462 tgaattggatccaggggacctgggctcattcttcttggctcaccaaccct 2511

2450 gcaggcctcatctttcccaaaacccactttgtcttggtgggagtgggtcc 2499
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2512 gcaggcctcatctttcccaaaacccactttgtcttggtgggagtgggtcc 2561

2500 gcgctgctctgcagcaggggctggggagtggacagcatcaggtgggaaag 2549
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2562 gcgctgctctgcagcaggggctggggagtggacagcatcaggtgggaaag 2611

2550 tggagtccaccctcatgtttctgtaggattctcaccgtggggctggaaga 2599
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2612 tggagtccaccctcatgtttctgtaggattctcaccgtggggctggaaga 2661

2600 aaagagcatcgacttgatttctccaaccactcatccctctttttctttct 2649
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2662 aaagagcatcgacttgatttctccaaccactcatccctctttttctttct 2711

2650 tccaccactccccaccccagctgtagttaatttcagtgccttacaaatcc 2699
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2712 tccaccactccccaccccagctgtagttaatttcagtgccttacaaatcc 2761

2700 taagctcagagaaagttccatttccgttccagagggaagggaacctccct 2749
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2762 taagctcagagaaagttccatttccgttccagagggaagggaacctccct 2811

2750 aggtccttccctggcttgttataacgcaaagcttggttgtttatgcaact 2799
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2812 aggtccttccctggcttgttataacgcaaagcttggttgtttatgcaact 2861

2800 ctatcttaagaactgcccagcctcagctgaaaacccgaatctgagaagga 2849
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2862 ctatcttaagaactgcccagcctcagctgaaaacccgaatctgagaagga 2911

2850 attgcgtcatgtaagggaagctggaattaagggagctgagccagtcatgg 2899
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2912 attgcgtcatgtaagggaagctggaattaagggagctgagccagtcatgg 2961

2900 ttgtggcgtgtgagtcaggagacctaggtttcagcccctctctactgtca 2949
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2962 ttgtggcgtgtgagtcaggagacctaggtttcagcccctctctactgtca 3011

2950 gcgagctgtgcaacgtgggcaagtcattgtcctctgagctgcagtttcct 2999
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3012 gcgagctgtgcaacgtgggcaagtcattgtcctctgagctgcagtttcct 3061

3000 catctgtcacatcgctacagacaagacctccctggaacccttctgattgt 3049
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3062 catctgtcacatcgctacagacaagacctccctggaacccttctgattgt 3111

3050 cttagacactgtggttgcaaaacccacggaaagcctcatttgtgtggaaa 3099
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3112 cttagacactgtggttgcaaaacccacggaaagcctcatttgtgtggaaa 3161

3100 gtcagaggaaaaatgatccagtggacacttggggattatctgtcattcaa 3149
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3162 gtcagaggaaaaatgatccagtggacacttggggattatctgtcattcaa 3211
```

```
3150 gatccttccttcaaccccaaggccagctcccatctcatttccagaaaggc 3199
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3212 gatccttccttcaaccccaaggccagctcccatctcatttccagaaaggc 3261

3200 tcatacctggcttgcagggaagcatctgtcttgtcattccaggtgccaga 3249
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3262 tcatacctggcttgcagggaagcatctgtcttgtcattccaggtgccaga 3311

3250 atcctctcagagtcattgaagggtgttcacccatcccacccaaggcttgg 3299
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3312 atcctctcagagtcattgaagggtgttcacccatcccacccaaggcttgg 3361

3300 cacactgccagtgtcttagcagggtcttgtgagggctgggggcatccagg 3349
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3362 cacactgccagtgtcttagcagggtcttgtgagggctgggggcatccagg 3411

3350 cactcagaaggcaaaggaaccaccctacccatttggcctctggaggggggc 3399
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3412 cactcagaaggcaaaggaaccaccctacccatttggcctctggaggggggc 3461

3400 agaagaaagaaagaaacctcatcctatattttacaaagcatgtgaattct 3449
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3462 agaagaaagaaagaaacctcatcctatattttacaaagcatgtgaattct 3511

3450 ggcattagctctcataggagacccatgtgcttccttgctcagtgcaaaac 3499
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3512 ggcattagctctcataggagacccatgtgcttccttgctcagtgcaaaac 3561

3500 tgatgattctacttgctgtagatgaatggttaacacgagctagttaaaca 3549
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3562 tgatgattctacttgctgtagatgaatggttaacacgagctagttaaaca 3611

3550 gtgccattgttttgccagtgaagcctccaaccctaagccactgggacggt 3599
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3612 gtgccattgttttgccagtgaagcctccaaccctaagccactgggacggt 3661

3600 ggccagagatgccagcagcctctgtcgcccttagtcatataaccaaaatc 3649
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3662 ggccagagatgccagcagcctctgtcgcccttagtcatataaccaaaatc 3711

3650 cagaccttatccacaacccggggcttggaaaggaaggtattttggaatca 3699
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3712 cagaccttatccacaacccggggcttggaaaggaaggtattttggaatca 3761

3700 caccctccggttatgttgctccagtaaaatcttgcctggaaagaggcagt 3749
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3762 caccctccggttatgttgctccagtaaaatcttgcctggaaagaggcagt 3811

3750 cttcttagcatggtgagctgagttcatggctttttttgtagccagtcct 3799
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3812 cttcttagcatggtgagctgagttcatggctttttttgtagccagtcct 3861

3800 gtccctggccatccatgtgatggttttggatggagttaaacttgatgcca 3849
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3862 gtccctggccatccatgtgatggttttggatggagttaaacttgatgcca 3911

3850 gtgggcagtgcatgtggaaagtatcagagtaagcctctcccctccagagc 3899
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3912 gtgggcagtgcatgtggaaagtatcagagtaagcctctcccctccagagc 3961

3900 cctgagtttcttggctgcatgaaggttttctttagaatcagaattgtagc 3949
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
3962 cctgagtttcttggctgcatgaaggttttctttagaatcagaattgtagc 4011
```

```
                .         .         .         .         .
3950 cagtttctttggccagaaggatgaatacttggatattactgaaagggagg 3999
     ||||||||||||||||||||||||||||||||||||||||||||||||||
4012 cagtttctttggccagaaggatgaatacttggatattactgaaagggagg 4061
                .         .         .         .         .
4000 ggtggagatgggtgtggcagtgtatggtgtgtgatttttatttttcttctt 4049
     ||||||||||||||||||||||||||||||||||||||||||||||||||
4062 ggtggagatgggtgtggcagtgtatggtgtgtgatttttatttttcttctt 4111
                .         .         .         .         .
4050 tggtcatgggggccaaggagaaaggcatgaatcttccctgtcaggctctt 4099
     ||||||||||||||||||||||||||||||||||||||||||||||||||
4112 tggtcatgggggccaaggagaaaggcatgaatcttccctgtcaggctctt 4161
                .         .         .         .         .
4100 acagccacaggcactgtgtctactgtctggaagacatgtccccgtggctg 4149
     ||||||||||||||||||||||||||||||||||||||||||||||||||
4162 acagccacaggcactgtgtctactgtctggaagacatgtccccgtggctg 4211
                .         .         .         .
4150 tggggccgctgcttctgtttaaataaaagtggcctgg 4186
     ||||||||||||||||||||||||||||||||||||
4212 tggggccgctgcttctgtttaaataaaagtggcctgg 4248
```

Fig. 16


1 MEPAVSLAVC ALLFLLWVRL KGLEFVLIHQ RWVFVCLFLL PLSLIFDIYY
51 YVRAWVVFKL SSAPRLHEQR VRDIQKQVRE WKEQGSKTFM CTGRPGWLTV
101 SLRVGKYKKT HKNIMINLMD ILEVDTKKQI VRVEPLVTMG QVTALLTSIG
151 WTLPVLPELD DLTVGGLIMG TGIESSSHKY GLFQHICTAY ELVLADGSFV
201 RCTPSENSDL FYAVPWSCGT LGFLVAAEIR IIPAKKYVKL RFEPVRGLEA
251 ICAKFTHESQ RQENHFVEGL LYSLDEAVIM TGVMTDEAEP SKLNSIGNYY
301 KPWFFKHVEN YLKTNREGLE YIPLRHYYHR HTRSIFWELQ DIIPFGNNPI
351 FRYLFGWMVP PKISLLKLTQ GETLRKLYEQ HHVVQDMLVP MKCLQQALHT
401 FQNDIHVYPI WLCPFILPSQ PGLVHPKGNE AELYIDIGAY GEPRVKHFEA
451 RSCMRQLEKF VRSVHGFQML YADCYMNREE FWEMFDGSLY HKLREKLGCQ
501 DAFPEVYDKI CKAARH

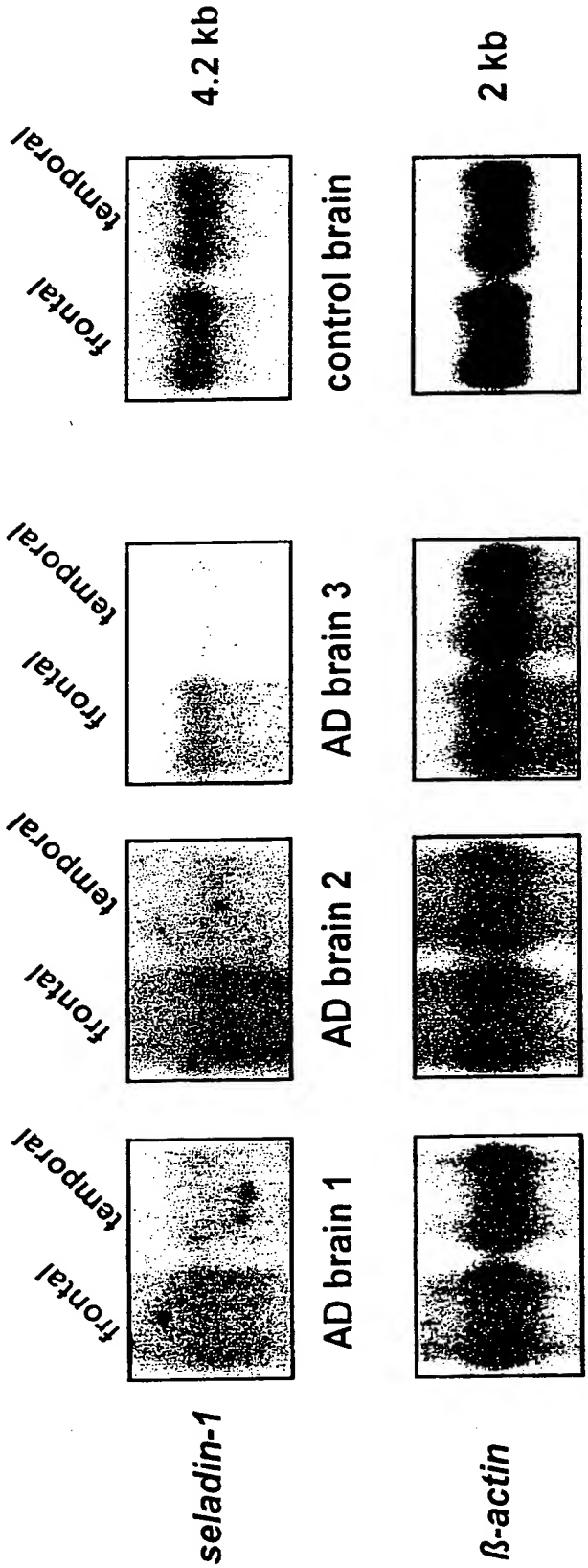

4.2 kb
2 kb
frontal
temporal
control brain
AD brain 3
AD brain 2
AD brain 1
seladin-1
ß-actin

Fig. 17 A

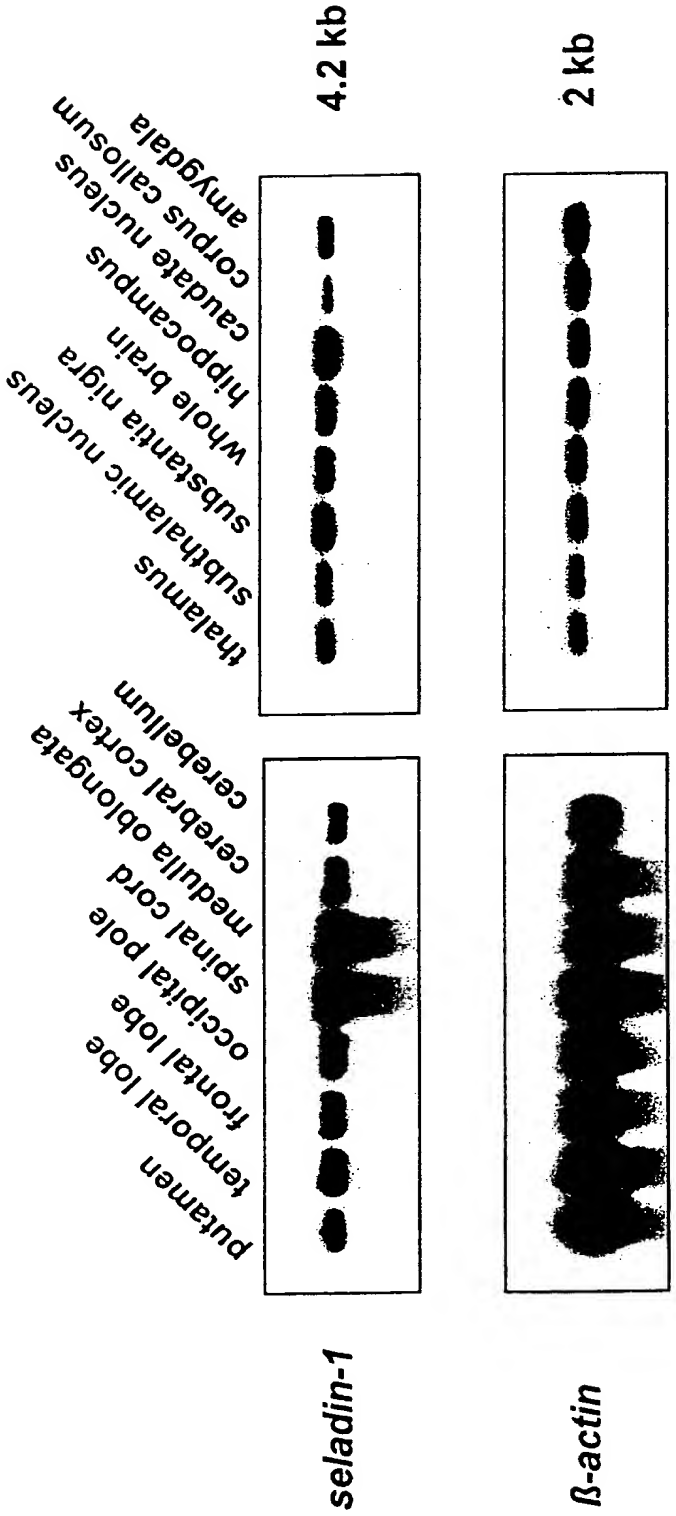

4.2 kb
2 kb
putamen
temporal lobe
frontal lobe
occipital pole
spinal cord
medulla oblongata
cerebral cortex
cerebellum
thalamus
subthalamic nucleus
substantia nigra
whole brain
hippocampus
caudate nucleus
corpus callosum
amygdala
seladin-1
β-actin

Fig. 17 B

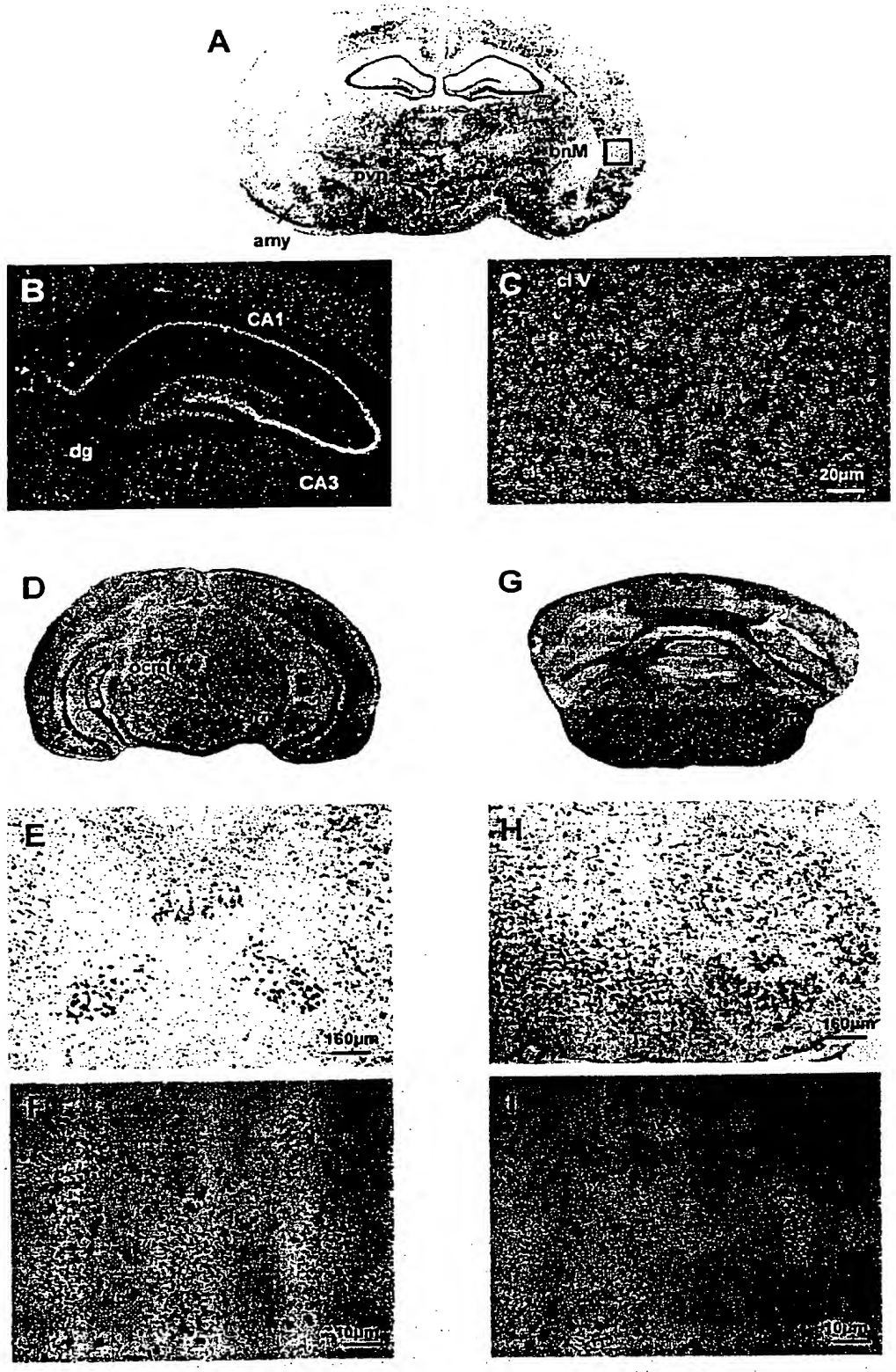

A
bnM
pvn
amy
B
CA1
dg
CA3
C
clV
20µm
D
cpnt
G
E
160µm
H
160µm
F
10µm
I
10µm

Fig. 18

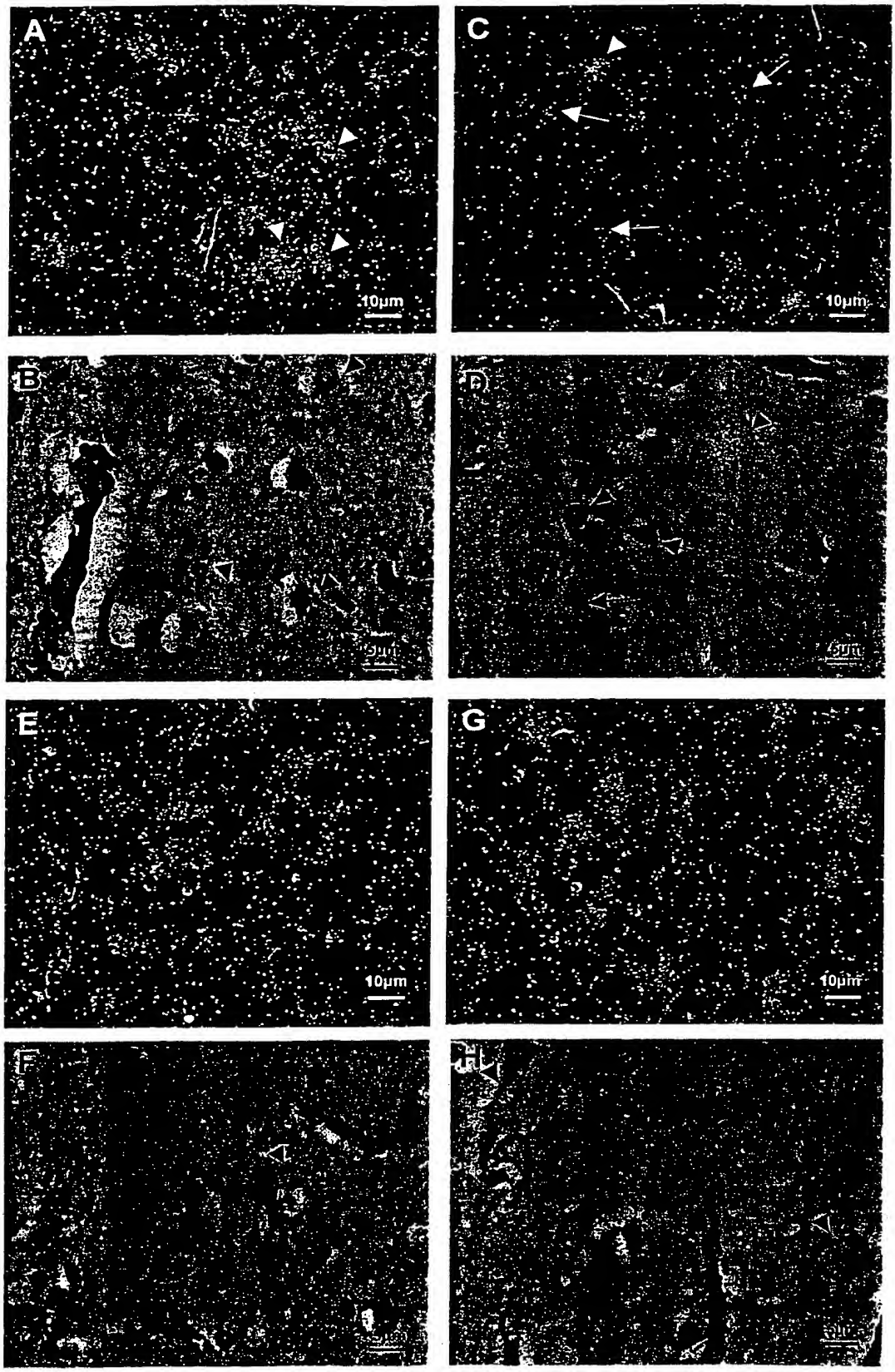


Fig. 19

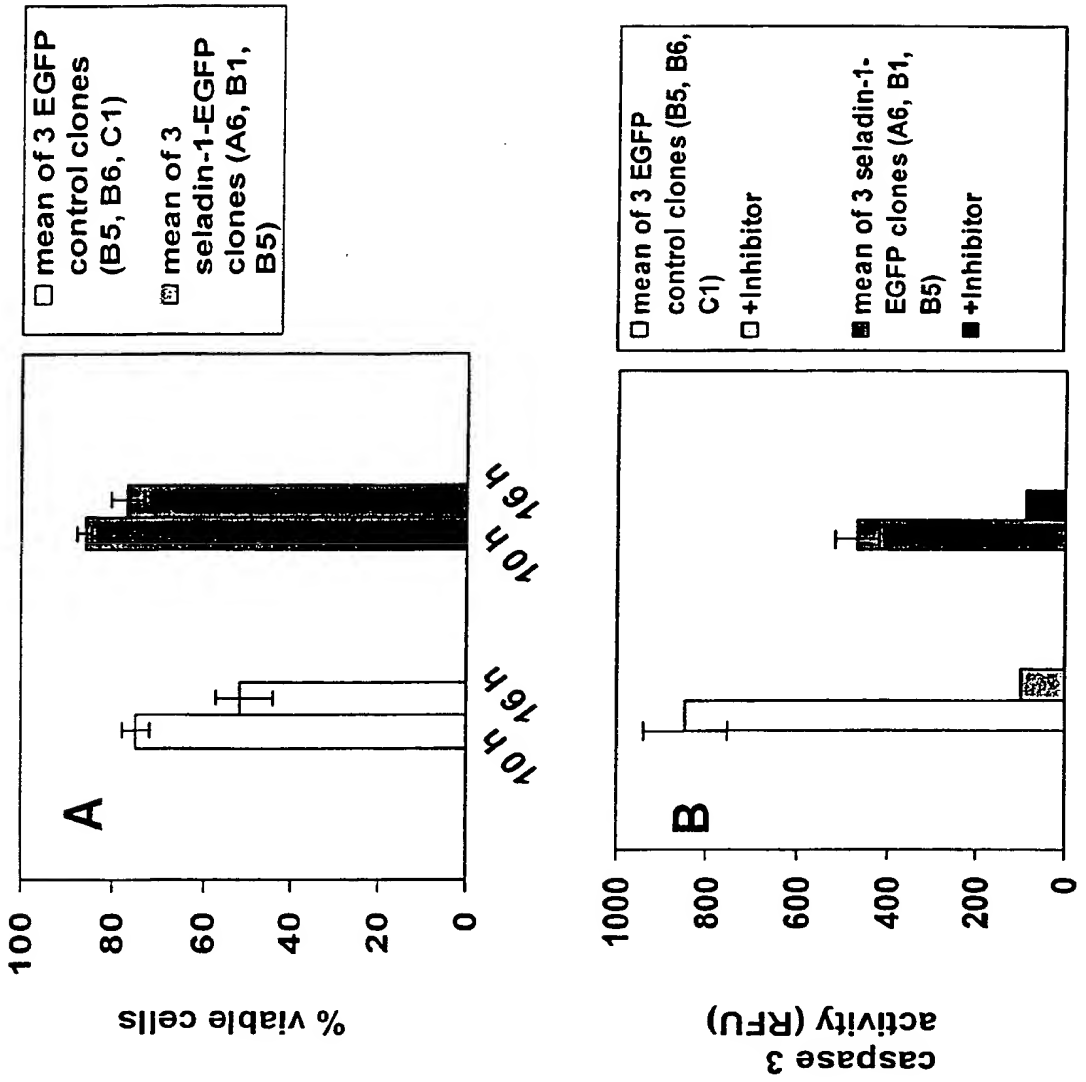

A
% viable cells
100
80
60
40
20
0
10h
16h
10h
16h
mean of 3 EGFP control clones (B5, B6, C1)
mean of 3 seladin-1-EGFP clones (A6, B1, B5)
B
caspase 3 activity (RFU)
1000
800
600
400
200
0
mean of 3 EGFP control clones (B5, B6, C1)
+Inhibitor
mean of 3 seladin-1-EGFP clones (A6, B1, B5)
+Inhibitor

Fig. 20

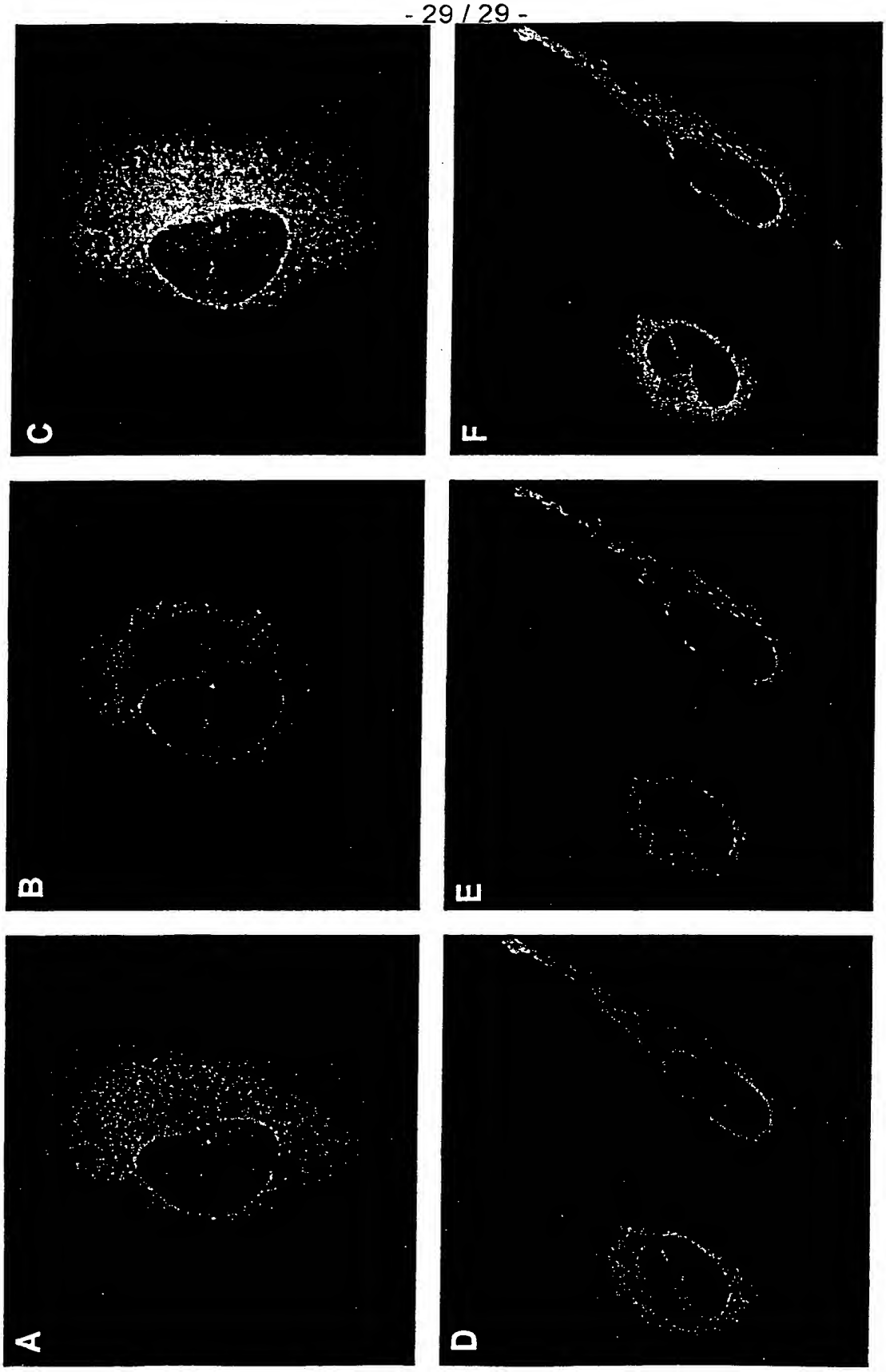


Fig. 21

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
992514wo Me/kk		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/ 08744	12/11/1999	12/11/1998
Applicant		
NITSCH, ROGER		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 5

   [X] as suggested by the applicant.

   [ ] None of the figures.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/08744

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
28-34 and partially 21-27 and 35-38
See FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by th applicant's protest.

☐ N protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 28-34 and partially 21-27 and 35-38

Present claims 21-38 relate to a compound defined by reference to a desirable characteristic or property, namely affecting a level, an activity or both of a substance selected from the group of (a) to (f) as defined in the above mentioned claims. The claims cover all compounds having this characteristic or property, whereas the application does not provide support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for any specific example of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the products defined as (a) to (f) for claims 21-27 and 35-38, while no search has been carried out for claims 28-34.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]EP 99/08744

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/47 C12Q1/68 G01N33/68 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Emhum2 Database Entry Hsrsc390 Accession number D13643; 31 March 1993 NOMURA N.: "Human mRNA for KIAA0018 gene, complete cds." XP002099607 the whole document -& NOBUO NOMURA ET AL.: "Prediction of the coding sequences of unidentified human genes. I. The coding sequences of 40 new genes (KIAA0001-KIAA0040) deduced by analysis of randomly sampled cDNA clones from human immature myeloid cell line KG-1" DNA RESEARCH, vol. 1, no. 1, 1994, pages 27-35, XP002099608 abstract; tables 3,4 -& NOBUO NOMURA ET AL.: "Prediction of the coding sequences of unidentified human -/--	5,8-13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 March 2000	10/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	genes. I. The coding sequences of 40 new genes (KIAA0001-KIAA0040) deduced by analysis of randomly sampled cDNA clones from human immature myeloid cell line KG-1 (Supplement)" DNA RESEARCH, vol. 1, no. 1, 1994, pages 47-56, XP002065816 the whole document	
A	EP 0 814 157 A (SMITHKLINE-BEECHAM CORPORATION) 29 December 1997 (1997-12-29) page 3, line 40 -page 9, line 32	1-27, 35-38
T	GREEVE, I. (1) ET AL: "Expression of Seladin -1, a novel neuroprotective gene with homologies to oxido-reductases is associated with selective vulnerability in Alzheimer's disease." SOCIETY FOR NEUROSCIENCE ABSTRACTS, (1999) VOL. 25, NO. 1-2, PP. 546. MEETING INFO.: 29TH ANNUAL MEETING OF THE SOCIETY FOR NEUROSCIENCE, PART MIAMI BEACH, FLORIDA, USA OCTOBER 23-28, 1999 THE SOCIETY FOR NEUROSCIENC. , XP002134188	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
[redacted]EP 99/08744

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 814157 A	29-12-1997	JP 10210970 A	11-08-1998